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THE PLANT DISEASE REPORTER

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PLANT DISEASE EPIDEMICS
and
IDENTIFICATION SECTION

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The Plant Disease Reporter is issued as a service to plant pathologists throughout the United States. It contains reports, summaries, observations, and comments submitted voluntarily by qualified observers. These reports often are in the form of suggestions, queries, and opinions, frequently purely tentative, offered for consideration or discussion rather than as matters of established fact. In accepting and publishing this material the Plant Disease Epidemics and Identification Section serves merely as an informational clearing house. It does not assume responsibility for the subject matter.

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PLANT DISEASE EPIDEMICS AND IDENTIFICATION SECTION

Crops Research Division, Plant Industry Station, Beltsville, Maryland
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SUGAR BEET CURLY TOP DISEASE IN TURKEY

C. W. Bennett and Aziz Tanrisever[1]

Summary

Sugar beet plants were found by the junior author in 1955, near Eskisehir, Turkey that showed leaf curling, vein swelling, phloem necrosis, and other symptoms characteristic of curly top disease as it is known in the United States. Dried beet leaves were sent to the United States Agricultural Research Station at Salinas, California, through the Plant Quarantine Branch of the United States Department of Agriculture. Virus was transmitted by the beet leafhopper, Circulifer tenellus (Baker), to seedling sugar beet plants from extracts of these leaves. The virus proved to have essentially the same host range, insofar as tested, as the curly top virus and symptoms on all hosts were similar to those produced by curly top virus. Varieties of sugar beet differing in resistance to curly top showed the same order of resistance to the virus from Turkey as to the curly top virus. Evidence indicates that the virus recovered from beets from Turkey is a strain of the curly top virus. Since C. tenellus, the only known vector of the curly top virus, is a native of the Mediterranean area, it seems highly probable that the curly top virus may have originated there also and that both the virus and its vector were introduced into North America from that region.

INTRODUCTION

Oman (4) pointed out that the beet leafhopper, Circulifer tenellus (Baker), the only known vector of the curly top virus in North America, has no close relatives in the Western Hemisphere, and suggested that it probably was introduced into North America from the Mediterranean area. Young and Frazier (6) have given a summary of the known distribution of the genus, Circulifer, as well as extensive records concerning the occurrence of C. tenellus in the Mediterranean region. Frazier (2) found a leafhopper in French Morocco which appears to be identical with C. tenellus. Freitag, Frazier, and Huffaker (3) showed that the leafhopper found by Frazier in French Morocco will cross with C. tenellus and that it is able to transmit the curly top virus.

These developments emphasized anew the question of the origin of the curly top virus. Is it a virus endemic to western United States that happened to be transmissible by an introduced insect, or was the virus introduced along with its known vector, C. tenellus? If the virus is endemic in western United States, it obviously must, at one time at least, have had a vector other than C. tenellus to ensure its survival prior to the introduction of the Mediterranean species. No such vector has been found. If the curly top virus was introduced from the Mediterranean area, it would seem reasonable to expect that curly top should occur on sugar beet in that area. However, curly top has not been reported from any part of the world other than the Western Hemisphere.

In view of these facts, it was of considerable interest when the junior author, in connection with studies of yellows and other virus diseases of sugar beet in Turkey, found plants near Eskisehir in 1955 with crinkled leaves that had a "rubbery feel" and had spine-like outgrowths from the veins characteristic of those produced by curly top.

Three leaves from one of these diseased plants were sent to the senior author at the United States Agricultural Research Station, Salinas, California, for comparison with curly top. These leaves had been immersed in a formaldehyde solution before mailing, to destroy any virus they might carry, and were partially collapsed when received; however, they showed typical crinkling, vein swelling, and the presence of spine-like outgrowths from the veins. Transverse sections of veins showed phloem necrosis indistinguishable from that produced by curly top. Tanrisever (5) recently pointed out the similarity of gross symptoms to those produced by curly top.

The similarities between symptoms of curly top on sugar beet in the United States and

1 Plant Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Salinas, California, and Pathologist, Turkiye Seker Fabrikalari A. S., Zirai Arastirma Laboratuarlari, Eskisehir, Turkey, respectively.

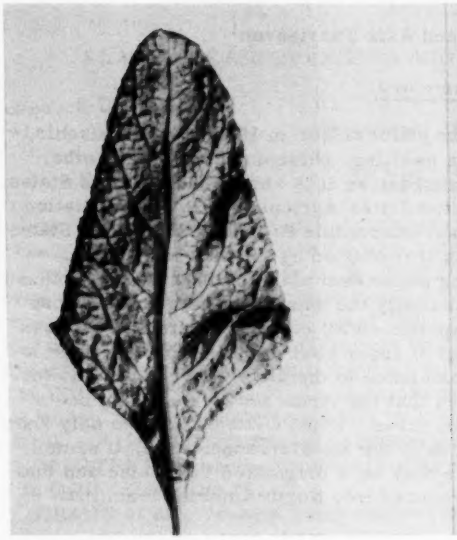

FIGURE 1. Sugar beet leaf showing vein swelling, vein distortion, and crinkling caused by North American curly top virus.

FIGURE 2. Sugar beet leaf showing vein swelling, vein distortion, and crinkling caused by Turkish curly top virus. (Photograph by Aziz Tanrisever).

symptoms caused by the disease in Turkey are illustrated in Figures 1 and 2. These figures show that leaf crinkling, vein swelling and distortion, and small protuberances on the veins are essentially the same on the leaf with North American curly top as on the leaf with the disease in Turkey.

The only disease of sugar beet reported from Europe, with which these symptoms might be confused, is "krauselkrankheit" transmitted by the tingid bug, Piesma quadrata Fieb. However, krauselkrankheit does not induce the development of spine-like outgrowths from the veins of leaves of diseased plants and there is more rolling and crinkling of leaves than observed on leaves of plants with the disease in Turkey.

RECOVERY OF VIRUS

After preliminary observations and tests had indicated a high degree of similarity between the leaf curling disease in Turkey and curly top in the United States, further attempts were made to identify the Turkish disease. The curly top virus has been shown to retain its activity in dried beet leaves for as long as 8 years (1). Therefore, leaves of infected sugar beet plants were dried by the junior author and sent to the United States Agricultural Research Station at Salinas, California, through the Plant Quarantine Branch of the United States Department of Agriculture, for tests to determine whether the beet leafhopper is able to act as a vector of the virus causing the disease in Turkey.

The dried leaves were ground in a mortar and soaked in distilled water which was extracted in a press after about 1 hour. The coarser particles were removed from the extract by centrifugation and the supernatant liquid was added to an equal volume of 95 percent ethyl alcohol. The resulting precipitate was removed by centrifugation, dried, and suspended in a solution of 6.8 mM sodium citrate to which enough sucrose had been added to make a 3 percent sugar solution. This was centrifuged and the supernatant liquid was fed to nonviruliferous beet leafhoppers through an animal membrane. After feeding, the leafhoppers were caged singly on seedling sugar beet plants.

Of 40 plants inoculated by this method, 7 showed distinct symptoms of disease characterized by veinlet clearing, vein swelling, and leaf rolling. Forty check plants, on which leafhoppers from the same source but which had not fed on liquid extract were caged, showed no evidence of infection.

Transfers were made from the infected plants to a number of varieties of sugar beet and to other species of plants and the effects of this virus were compared with those produced by a virulent strain (strain 3) of the curly top virus.

SYMPTOMS PRODUCED BY THE VIRUS FROM TURKEY

Beta vulgaris L. Sugar Beet -- Varieties very susceptible to curly top in the United States began to show distinct clearing of veins of young leaves 7 to 14 days after inoculation. Veins became swollen, leaves were curled and twisted, small drops of a clear liquid appeared on the blades and petioles, and there was considerable necrosis in the phloem of the petioles. Plants were not markedly dwarfed and a high degree of recovery followed the more severe initial symptoms. In the recovery stage leaves were more or less normal in shape and size but the veins showed some swelling and irregularity and produced marked spine-like outgrowths in some instances.

Eight selections of sugar beet, ranging in curly top susceptibility from very susceptible (S. L. 842) to very resistant (S. L. 96), were inoculated with the virus from Turkey and their reactions compared with those produced by strain 3 curly top virus. Symptoms produced by strain 3 were more severe than those produced by the Turkish virus, but the selections showed the same order of resistance to each virus. The selection S. L. 96, on which strain 3 produced only mild symptoms, showed no indication of infection following inoculation with the Turkish virus.

Nicotiana tabacum L. Tobacco, var. Turkish -- Symptoms were very mild even on seedling plants and consisted of inconspicuous vein clearing or yellowing and hardly perceptible rugoseness of mature leaves. Shoots developed from cuttings of infected plants showed more obvious vein clearing of young leaves than seedling plants.

Distinct swelling of veins was produced on the corollas of flowers of plants that blossomed. Symptoms produced by this virus were milder than those produced by any known strain of curly top virus that infects tobacco. Most of the less virulent strains of curly top virus, however, do not infect Turkish tobacco.

Nicotiana glutinosa L. -- Symptoms were very severe on plants of this species when infection was produced by feeding of the beet leafhopper. Leaves were severely curled and dwarfed and had a deeper green color than normal. Older leaves died prematurely, the plants were dwarfed, and some of the plants died 4 to 6 weeks after infection. Some of those that survived sent out axillary shoots that were nearly normal in appearance and the plants produced a high degree of recovery. Symptoms were less severe in plants inoculated by means of scions from infected tomato plants than in plants inoculated by means of the beet leafhoppers. This type of partial protection is found also with American strains of curly top virus on this species.

Lycopersicon esculentum Mill. Tomato -- Symptoms were very mild on tomato plants inoculated either by means of leafhoppers or by graftage. Young plants inoculated by means of leafhoppers showed inconspicuous vein clearing, and a slight amount of curling and dwarfing but the plants were not markedly stunted. Symptoms were difficult to distinguish on plants that were growing rapidly.

Other Susceptible Species -- In addition to the plants already named, the virus from Turkey was transmitted to Beta macrocarpa Guss., Chenopodium album L. (lamb's quarters), C. amaranticolor Coste & Reyn., C. murale L. (sowbane), Datura stramonium L. (jimson weed), Malva parviflora L. (cheese-weed), X Petunia hybrida Vilm. (petunia), Nicotiana glauca Graham (California tree tobacco), Stellaria media (L.) Cyr. (chickweed), and Zinnia elegans Jacq. (zinnia).

Nicotiana glauca is a symptomless carrier of the virus as is true with all of the tested strains of the curly top virus. On the other species, symptoms were less severe than those produced by strain 3, except on Chenopodium murale. This species is very resistant to all of the strains of curly top virus tested in the United States and it appears to be immune to most

of them including several of the virulent strains. The Turkish virus produced marked vein swelling and leaf curling and plants were dwarfed.

DISCUSSION AND CONCLUSIONS

The results obtained in studies of the virus from Turkey indicates very strongly that this virus is a strain of the curly top virus complex and that it is closely related to curly top virus that has a wide distribution in western United States. Symptoms on all host plants tested are very similar to those produced by known strains of the curly top virus. Sugar beet varieties and selections with a wide range of susceptibility to curly top in the United States show the same order of resistance to the Turkish virus. This is true also for susceptible species of plants tested, with the exception of a single species, Chenopodium murale, which is more severely injured by the Turkish virus than by a virulent strain of curly top virus. C. murale, however, has shown greater susceptibility to infection with the less virulent than with the more virulent strains of curly top virus. The results, therefore, may not be greatly at variance with those obtained with strains of the curly top virus known to occur in the United States.

The Turkish virus is readily transmitted by the beet leafhopper, Circulifer tenellus. This insect is not known to be able to transmit any virus other than curly top virus, and it is the only known vector of the curly top virus. In most instances where extensive tests have been made, leafhopper-transmitted viruses have been shown to have more than one vector. Usually the vectors are related species. The fact that C. tenellus has no close relatives in North America may offer an explanation for its apparent specificity in transmission of curly top virus. It seems probable that further tests of species of leafhoppers related to C. tenellus may reveal additional vectors. The discovery of such vectors would provide further evidence pointing toward the Mediterranean area as the place of origin of the curly top virus and would support the concept that both the curly top virus and its known vector were introduced into the Western Hemisphere from the Mediterranean region.

If the Mediterranean area is the place of origin of the curly top virus it would appear that the curly top disease should have been discovered there at a much earlier date. It is probable, however, that there may not be many host plants of economic importance in the area on which symptoms of the disease may be readily identified. Also, Frazier (2) states that the beet leafhopper was not found on sugar beet in French Morocco although it was found on other species of plants. It would seem probable, therefore, that incidence of infection in beet fields in past years may have been low. This, together with the occurrence of strains of virus that produce relatively mild symptoms, associated with a considerable degree of recovery, may account for past failures to identify the disease.

The strain of virus recovered from Turkish beet plants is not highly virulent and probably would not cause extremely heavy losses. It is possible, however, that more virulent strains may occur. If so, curly top could become a serious threat to an industry based on curly top susceptible varieties. Results thus far with the virus from Turkey indicate that the disease in the Mediterranean area could be controlled readily by use of varieties already developed in the United States for resistance to the curly top disease. Field tests with these varieties would be desirable to determine adaptation to local conditions.

Acknowledgment

The writers are indebedted to Phyllis R. Emparan of the United States Agricultural Research Station, Salinas, California, for valuable assistance with all of the transmission work.

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A NEW VIRUS DISEASE OF COTTON IN TEXAS

David W. Rosberg[1]

During the past 4 years, cotton research workers have reported the occurrence of an occasional cotton plant in experimental and commercial plantings in the Lower Rio Grande Valley that exhibited virus-like symptoms. On several occasions in 1955, leaf specimens sent to the writer were processed and inoculations made with expressed leaf sap to cotton, bean, cowpea, tobacco, and hollyhock plants. Symptoms failed to develop following any of these attempts.

In 1956, a considerable number of cotton plants in the greenhouse and screenhouse of the United States Pink Bollworm Research Center, Brownsville, Texas developed virus-like symptoms typical of those previously observed.

Approach grafts of diseased to healthy plants of the Empire variety were made[2]. Approximately 9 weeks after grafting, the healthy Empire plants developed leaf symptoms typical of those of the diseased cotton plant to which it was grafted. Young leaves immediately above the graft union were the first to exhibit symptoms. Symptoms developed in the terminal leaves of the plants 7 to 14 days later.

The disease symptoms are characterized by a pronounced veinclearing of affected older leaves, accompanied by the development of chlorotic areas at the tips of smaller veins. Veinbanding, intervenal yellowing, and mottling gradually develop. Young terminal leaves first exhibit spotted intervenal yellowing and a slight to severe downward cupping. Frequently, young leaves exhibit typical mosaic symptoms and puckering. Both young and older leaves develop intervenal red-spotting of varying intensity, followed by a general and gradual degeneration of the chlorophyll. At this stage, the leaves become more or less tan to buff-colored, red-spotting is pronounced, and the leaves abscise readily (Figure 1). Infected plants are somewhat stunted, and terminal internodes are shortened. The bolls are one-half to two-thirds normal size, with mottled bracts containing small and poorly-filled seeds (Figure 2).

Leaves from diseased plants were harvested and processed for electron microscopy studies by alternate high and low speed centrifugation of expressed leaf sap in a Servall SS-2 centrifuge over a range of 6,000 xg and 62,000 xg for 2 to 8 hours. The final precipitates were suspended in distilled water, serially diluted, sprayed on the surface of a Formvar-covered specimen screen, gold shadowcasted, and examined in an RCA EMU-type Electron Microscope. Electron micrographs of the virus (Figure 3A) show the virus particles to be spherical in shape and approximately 30 mμ in diameter. An unidentified proteinaceous substance was found to be associated with the virus. Often, masses of virus particles were embedded within this substance (Figure 3B).

To determine whether the virus is carried over in seed, 400 seed harvested from diseased plants were planted and observed for the development of symptoms. Resulting seedlings were free from symptoms, suggesting that the virus is not seed-transmitted.

On March 21, 1957 a survey was made of the screenhouse and outdoor Malvaceae garden of the United States Pink Bollworm Research Center at Brownsville, Texas for the incidence of virus disease in cotton. A close examination revealed that practically 100 percent of the Deltapine and Empire cotton plants (an estimated 5,000 plants) in the screenhouse covering approximately one-half acre were infected with the cotton virus. In addition, plants of six other species of Gossypium, six races of Gossypium hirsutum, and two Gossypium crosses, as well as a considerable number of okra plants, exhibited virus symptoms typical of those observed in Deltapine and Empire cotton. Over the past 9 months, an estimated 300 virus-infected cotton plants have been discarded from the greenhouse.

The following is a list of species of the Malvaceae which were found in this survey to exhibit symptoms of virus infection typical of those in Empire and Deltapine cotton plants employed in transmission studies[3].

---

[1] Associate Professor, Department of Plant Physiology and Pathology, Texas Agricultural Experiment Station, College Station, Texas.

[2] Diseased plants for virus transmission studies were furnished by personnel of the United States Pink Bollworm Research Center, Brownsville, Texas.

[3] Plant identifications were made by Mr. Ivan Shiller, Entomologist of the Pink Bollworm Research Center, Brownsville, Texas.

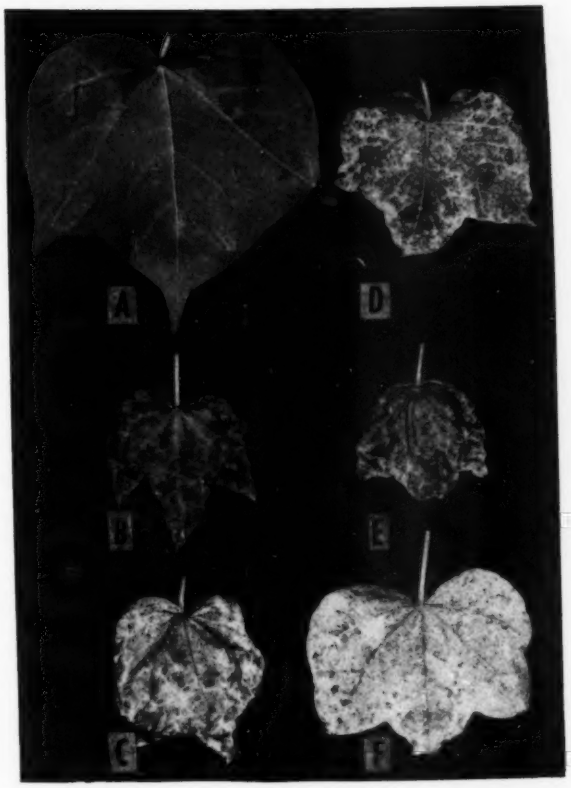

FIGURE 1. A. Healthy leaf. B. Young terminal leaf showing veinclearing and mottling. C. Young leaf showing intervenal yellowing and downward cupping. D. Older leaf, veins cleared and showing intervenal yellowing. E. Older leaf showing mottling and distortion due to shortened veins. F. Old leaf, buff colored with intervenal red spots.

FIGURE 2. A. Healthy boll. B. Boll from virus-infected plant showing mottled bracts.

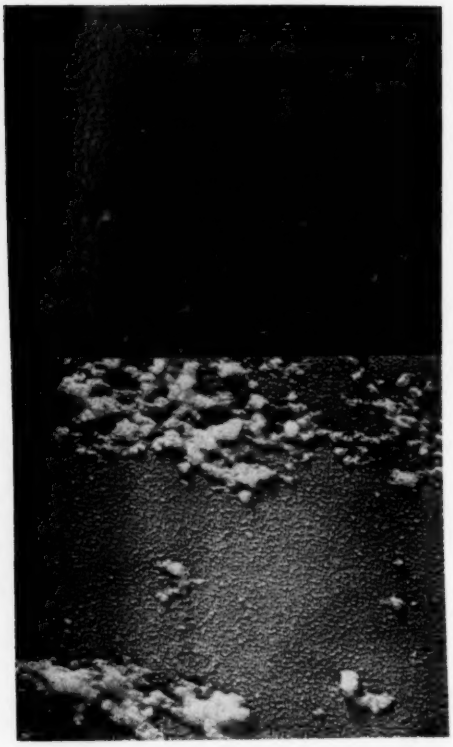

FIGURE 3. Electron micrographs of cotton virus. A. Preparation air dried from a distilled water suspension (X about 45,000). B. Air dried distilled water preparation. Note virus particles embedded in proteinaceous substance (X about 19,000).

Gossypium hirsutum, race palmeri
Gossypium hirsutum, race morrilli
Gossypium hirsutum, race richmondi
Gossypium hirsutum, race punctatum
Gossypium hirsutum, race marie-galante
Gossypium hirsutum, race latifolium
Gossypium arboreum
Gossypium barbadense
Gossypium anomalum
Gossypium thurberi
Gossypium davidsonii
Gossypium ramondii
Gossypium hirsutum X Gossypium herbaceum
Gossypium hirsutum X Gossypium sturtii
Hibiscus esculentus L. (Okra)

In many respects, symptoms of the Texas cotton virus disease resemble those of leaf curl disease of cotton reported in Sudan and Nigeria (1, 2) and leaf crumple virus disease of cotton in California (3). However, veinbanding, conspicuous red spotting, and defoliation of older buff-colored leaves are not reported as symptoms of these diseases. Further, transmission of the virus by grafting to Hibiscus cannabinus L., which is susceptible to the African leaf curl virus, has not been successful.

Transmission studies employing several methods of grafting and mechanical inoculation techniques to various species of plants are in progress in an effort to establish the identity of the Texas cotton virus. The serological relationships of the virus also will be investigated.

Acknowledgment

The cooperation of Dr. S. E. Jones and Messrs. M. J. Lukefahr, C. A. Richmond, and Ivan Schiller of the United States Pink Bollworm Research Center, Brownsville, Texas, in this study is gratefully acknowledged.

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HOST RANGE OF TWO STRAINS OF THE CEREAL YELLOW-DWARF VIRUS[1]

G. W. Bruehl[2] and H. V. Toko[3]

Summary

A host range study of two Washington strains of cereal yellow-dwarf virus showed that many grasses are hosts, but that considerable variation in host range exists, both between the Washington strains and in comparison with the host range reported in California. Examples of these variations in host range are smooth brome (Bromus inermis), immune to both Washington strains, susceptible in California; timothy (Phleum pratense), susceptible to both Washington strains, immune in California; and Poa spp., susceptible to one Washington strain, immune to the other.

Oswald and Houston, in their original pioneering work on the subject, discovered that the cereal yellow-dwarf virus has an extensive host range among grasses (3). They stressed the importance of these many hosts as a more or less permanent source of virus. Two Washington collections of the cereal yellow-dwarf virus were studied on several grasses to determine how closely their host ranges would agree with each other and with that found in California.

The two Washington collections were: the "original" Washington strain (2) of cereal yellow-dwarf from a weedy roadside grass near Walla Walla, Washington, fall, 1954; and the "English grain" strain (6), from oats near Colfax, Washington, fall, 1955. The "English grain" strain is so-called because it was transmitted by the English grain aphid (Macrosiphum granarium Kirby), and not by the apple grain aphid (Rhopalosiphum fitchii Sand.). The "original" Washington strain was transmitted by both aphids, was more virulent on oats, wheat, and barley in greenhouse trials, and is considered more typical.

Seed of the grasses tested was sown in steamed soil in 4-inch clay pots in the greenhouse. When the grasses were 1 to 2 inches high, viruliferous aphids from diseased oats were placed upon them for at least 48 hours and then removed by fumigation with Nicofume. Both species of aphids were used with the original strain; English grain aphids were used with the English grain strain. The grasses were then kept aphid-free. Grasses were subsequently observed for symptoms.

Non-viruliferous aphids were then placed on the grasses to attempt recovery of virus after incubation of a month or more. The apple grain aphid was used in recoveries of the original Washington strain, and the English grain aphid for the English grain strain. In recovery trials the non-viruliferous aphids were allowed 48 hours or more for accession and a 48-hour transmission period on seedling Fulghum oats. The insects were then removed by fumigation. Checks, as well as the many negative attempts, validated the results. The development of yellow-dwarf on the oats was taken as proof that the particular grass was a host.

The data (Table 1) show that each of the virus collections has a wide host range. Results of the California study (3) are included to permit comparison. The host ranges of the three collections of virus vary considerably. All wild Bromus and Hordeum species tested were hosts. These grasses are common along roadsides and other uncultivated places in Washington, and they are good food plants for both the apple grain and English grain aphids, our most prevalent cereal aphids.

Smooth brome (Bromus inermis) is a noteworthy exception. This valuable grass is immune to both Washington isolates but is susceptible in California (3). Timothy (Phleum pratense), reported as immune in California, was infected by the Washington isolates. The original Washington collection infected all Poa spp. tested, but the English grain strain infected

[1] Scientific paper No. 1643, Washington Agricultural Experiment Stations, Pullman; Project No. 1280. Acknowledgment is gratefully made to P. M. Halisky for initial work on this study, and to J. P. Meiners for seeds of many species.

[2] Associate Pathologist, Washington State College, Pullman, Washington.

[3] Formerly Research Assistant, Washington State College, now Pathologist, USDA, Potato Handling Research Center, Presque Isle, Maine.

none. Table 1 shows that generalization as to host range is dangerous.

Qualitative differences also exist. The original Washington strain produced red-purple discoloration on all the wild bromes. Effects of the English grain strain ranged from symptomless, to red-purple discoloration, to dark green with marked leaf serration and severe stunting (the latter symptoms on Bromus commutatus and B. secalinus).

Setaria viridis became red-purple following the feeding of viruliferous aphids, but all attempts to recover virus from this grass failed.

To be important in epiphytology, the host species must not only be susceptible to the virus, but must also be a suitable feeding plant for the vector. The English grain aphid fed readily upon all the grasses used and multiplied on most of them. The apple grain aphid, in contrast, definitely disliked many grasses and would not multiply on a large number of them. Palatability of the grass is particularly important, because these vectors cannot become infective unless they feed on the source plant for about a day.

A conclusion that these host range studies distinguish two strains of a given virus is subject to doubt on two important points. The question arises: are atypical isolates merely strains of the cereal yellow-dwarf virus, or are they different viruses entirely? Both Washington strains compared here produced typical symptoms of the yellow-dwarf disease in accordance with the symptoms carefully described by Oswald and Houston on oats, wheat and barley. In addition, even though the host ranges differ, the symptoms produced upon the many grasses are typical of yellow-dwarf in its several manifestations. It seems unlikely that different viruses would reproduce a syndrome on so many different hosts. From a practical standpoint, it is better to consider both strains as belonging to the cereal yellow-dwarf virus, at least until some better means of distinction is found.

Another weakness in the distinction between strains of yellow-dwarf virus is that the grasses tested were not identical with those used in California. Strains of a given grass may vary in reaction to yellow-dwarf. Oswald and Houston (3) found that Double Dwarf 38 milo and Double Dwarf White Sooner milo (strains of Sorghum vulgare Pers.) differed in susceptibility. The former is a symptomless carrier whereas the latter is immune. Doubtless such differences could exist in other grasses. For the most part, however, the differences in host range are considered real. All varieties of oats, wheat, and barley tested so far show some degree of symptom expression. It seems unlikely that species with as much variation as these cereals should be alike in being susceptible, and that many other grasses should contain sufficient divergence in susceptibility to limit the value of these results seriously.

Most studies of variation in virulence within a pathogenic entity concentrate on reactions of agronomic varieties of the major hosts to the various collections. In cereal yellow-dwarf, strains of the virus determined by differential reactions on varieties of oats, wheat, or barley, have been reported (1, 2, 5). This first attention to varietal reaction results from practical considerations. Before varieties may be recommended as resistant, their response to the several strains of the yellow-dwarf virus probably prevalent in the area must be known.

Evidence is accumulating that strain differences in yellow-dwarf exceed the usual varietal reactions in that they extend to species and even generic levels. Summers and Bowman (4) found a red-leaf disease of oats in Mississippi. They were able to produce this disease in oats by means of viruliferous aphids, but could not infect Hardired winter wheat. Slykhuis, according to Watson and Mulligan (7), obtained the yellow-dwarf virus from timothy (Phleum pratense) and perennial ryegrass (Lolium perenne) in England. These grasses were immune to the strain tested by Oswald and Houston (3). Wit (8) encountered a problem as to the nature of a disease of perennial ryegrass in the Netherlands: "According to Oswald and Houston Lolium perenne should be immune from yellow-dwarf.... Either the yellow-dwarf virus encountered in the Netherlands is more virulent to perennial ryegrass than the California strain, or the virus is the same but the material tested by Oswald and Houston happened to be immune.....".

The present authors' results, coupled with observations of other investigators, indicate that the yellow-dwarf virus exhibits unusual variation. Not only do various strains affect agronomic varieties differentially, but they may have diverse host ranges. Possibly these host ranges vary in accord with the complex of host species present in a given locality. Species relished by vectors may be particularly influential, providing maximum opportunity for a given strain to persist and increase in that host complex.

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Table 1. Host ranges of three strains of the cereal yellow-dwarf virus.

Host	Virus strain		
	Washington		California[a]
	Original	English grain	
Agropyron amurense Drobov	-[b]		
A. cristatum (L.) Gaertn.	-	-	-
A. elongatum (Host) Beauv.		-	
A. inerme (Scribn. & Smith) Rydb.	+	-	
A. intermedium (Host) Beauv.	++	-	
A. repens (L.) Beauv.	-	-	
A. sibiricum (Willd.) Beauv.		-	
A. smithii Rydb.	-		
A. trachycaulum (Link) Malte		-	+
A. trichophorum (Link) Richt.	+	-	
Agrostis alba L.	-		-
A. exarata Trin.			-
A. palustris Huds.		-	
Alopecurus arundinaceus Poir	-	-	
A. pratensis L.		-	-
Arrhenatherum elatius (L.) Presl.		-	-
Avena fatua L.	++	++	++
A. sativa L.	++	++	++
Beckmannia syzigachne (Steud.) Fernald	+	-	
Bromus brizaeformis Fisch. & Mey.	++	++	
B. carinatus Hook. & Arn.	++	++	
B. catharticus Vahl	++	++	++
B. commutatus Schrad.	++	++	
B. erectus Huds.	++	++	
B. inermis Leyss.	-	-	++
B. japonicus Thunb.	++		
B. mollis L.	++	++	++
B. racemosus L.	++	++	
B. rigidus Roth.	++	++	++
B. rubens L.			++
B. secalinus L.	++	++	
B. sterilis L.		++	
B. tectorum L.	++	++	
B. tomentellus Boiss.		+	
Dactylis glomerata L.	+	++[c]	+
Deschampsia caespitosa (L.) Beauv.	+	-	
D. danthonioides (Trin.) Munro ex Benth.		+	-
Echinochloa crusgalli (L.) Beauv.		-	-
Elymus canadensis L.	-	-	
E. caput-medusae L.	+		+
E. condensatus Presl.	+	-	
E. giganteus Vahl		-	+
E. glaucus Buckl.		-	-
E. junceus Fisch.		-	
E. triticoides Buckl.		-	+

Table 1. (Continued.)

Host	Virus strain		
	Washington		California
	Original	English grain	
Festuca arundinacea Schreb.	+	+	+
F. elatior L.	+	+	
F. idahoensis Elmer	+	+	
F. myuros L.			++
F. ovina L.	+	+	
F. reflexa Buckl.		+	++
F. rubra L.	+	+	
Holcus lanatus L.	-	-	-
Hordeum brevisubulatum (Trin.) Lk.		+	
H. bulbosum L.		+	
H. leporinum Link	++	++	++
H. vulgare L.	++	++	++
Koeleria cristata L.	++	+	
Lolium multiflorum Lam.		+	+
L. perenne L.	-	++	-
L. remotum Schrank	++	++	
L. temulentum L.		++	
Oryzopsis hymenoides (Roem. & Schult.) Ricker		-	-
Phalaris arundinacea L.	+	+	
P. paradoxa L.			++
P. tuberosa L.		-	+
Phleum boehmeri		-	
P. phleoides (L.) Karst.		-	
P. pratense L.	+	++	-
Poa ampla Merr.	+	-	
P. annua L.			+
P. bulbosa L.		-	
P. canbyi (Scribn.) Piper	+	-	
P. compressa L.		-	
P. pratensis L.	+	-	+
Secale montanum Guss.		-	
Setaria lutescens (Weigel) F.T. Hubb		++	
S. viridis (L.) Beauv.	-	-	-
Sitanion hystrix (Nutt.) J.G. Smith		+	++
S. jubatum J.G. Smith		++	
Stipa cernua Stebbins & Love			-
S. comata Trin. & Rupr.		++	
Triticum aestivum L.	++	++	++

[a] California strain reactions taken from Oswald and Houston (3).

[b] Symbols: -, no virus was recovered, presumed immune; +, the virus was recovered, but the host remained essentially symptomless; ++, the virus was recovered and easily visible symptoms were produced.

[c] Ten lines of D. glomerata were tested with the original strain and no symptoms were produced; a Dwarf selection reacted to the English grain strain with typical mottle and chlorosis.

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BARLEY STRIPE MOSAIC VIRUS IN KANSAS AND ITS CONTROL BY A GREENHOUSE SEED-LOT TESTING TECHNIC[1]

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Abstract

Plants from a spring barley seed lot (probably variety Beecher) known to be heavily infected with barley stripe mosaic, a seed-borne virus disease (4), were grown during the late fall, winter, and early spring at light intensities varying from 800 to 10,000 foot candles and at temperatures ranging from 60° to 110° F. Optimum conditions found for symptom expression were high light intensities (10,000 foot candles) and temperatures of 70° to 85°. Even at high light intensities symptoms were diffuse, scarce, or absent in infected plants grown at temperatures below 70° and above 90°. At the optimum temperatures symptom expression was intermediate at 4000 foot candles and poor or absent at low light intensities of 3000 foot candles or less.

Four hundred eighteen farmers' seed samples from the 1955 and 1956 Kansas winter and spring barley crops were grown in the greenhouse at the optimum environmental conditions for symptom expression. Twenty-five seeds, selected at random, were grown from each seed sample. The virus was found to be widespread in Kansas, but most infected seed lots had a low percentage of infected seed. Total infection was 2.4 percent in all winter barley samples tested, with an average of 8.5 percent diseased plants in all infected samples. Of the 206 samples of winter barley tested, 63 contained infected seed. In spring barley seed the over-all infection for all samples was 2.8 percent, with an average of 8.9 percent diseased plants in all infected seed samples. Of the 212 samples of spring barley tested, 73 had infected seed.

According to results of cross inoculations from symptomless plants to wheat, barley, and corn, nearly all infected plants grown under favorable environmental conditions developed symptoms, which made cross inoculation of the samples unnecessary except in the case of possible very mild or entirely symptomless strains of the virus. When environmental conditions were optimum for symptom expression, such symptomless or very mild strains were not found as yet in any barley seed tested.

The technic described would be very satisfactory in eliminating heavily infected barley seed lots, although, if possible, it might be wise to use 100 seeds per sample in a seed certification program.

INTRODUCTION

Barley stripe mosaic (false stripe) is a seed-borne virus disease of barley which is known to cause considerable yield reduction in some varieties (1, 2, 5, 8). Attempts to control the disease by chemical seed treatment (1) and hot water seed treatment (6) have been ineffective, and although some tolerant varieties have been found (6, 8), good resistant varieties have not been reported. McKinney (6) thought that the disease might be controlled partially by seed indexing, and suggested possible cultural practices for detecting virus infected seed lots. In his report, the occurrence of symptomless carriers made seed indexing difficult, and cross inoculation of seedlings often was necessary to detect infected plants.

The purposes of this study were to determine the distribution and prevalence of the disease in Kansas, the effect of certain environmental conditions on seedling symptom expression in diseased plants grown from naturally infected seed, and to devise a greenhouse seed certification technic for detecting and eliminating the more heavily infected barley seed lots.

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Table 1. The effect of light and temperature on symptom development in barley stripe mosaic virus[a]

	Temperature °F				
	60	70	80	80-90	80-110
		800 foot candles			
Number of plants	—	160	—	139	—
Percent developing symptoms	—	15	—	16	—
		3000 foot candles			
Number of plants	128	47	193	—	—
Percent developing symptoms	7	17	15	—	—
		4000 foot candles			
Number of plants	—	—	92	—	—
Percent developing symptoms	—	—	20	—	—
		10,000 foot candles			
Number of plants	—	—	93	—	96
Percent developing symptoms	—	—	27	—	0

[a] The same infected spring barley seed lot was used in all tests. It was probably the variety Beecher but this is not certain as it was an unmarked farmer's seed sample. Two trials were completed at each temperature and light intensity in which data are given.

MATERIALS AND METHODS

Experiments were conducted to determine what environmental conditions were most favorable for symptom expression. Barley plants from a known heavily infected seed source were grown under different conditions of light and temperature. In the first experiment, three separate sets of plants were grown at 60°, 70°, and 80° F and at light intensities which ranged from 3000 foot candles on overcast days to as high as 10,000 foot candles on clear days. Cloudy days were predominant during this period, and light intensities higher than 3000 foot candles occurred only occasionally. The seedlings were grown individually in 3-inch pots on greenhouse benches and were observed daily for the appearance of symptoms. The experiments were repeated, using temperatures of 60°, 70°, and 85° for the three sets of plants.

In the second group of experiments two sets of plants from the same seed source used in the first experiments were grown at 70° and at 80° to 90° F but at a light intensity of only 800 foot candles. These plants were grown individually in 3-inch pots in environmental control chambers lighted with fluorescent and incandescent lamps. The photoperiod was maintained at 14 hours. The plants were grown for 14 days in the chambers and observed for symptoms. Then they were moved to an unshaded greenhouse bench where the light intensity often reached 10,000 foot candles and were observed 14 days for the appearance of additional symptoms at the higher light intensity. The experiment was then repeated.

In the third group of experiments plants from the same seed source mentioned previously were grown at various temperatures and at a light intensity of 10,000 foot candles. The experiments were conducted during March and April, 1956 and most of the days during this period

Table 2. Summary of the occurrence of barley stripe mosaic virus in seed of Kansas winter barley varieties.

	Reno	Dicktoo	B-400	Miscellaneous	Total
		1955			
Total number of samples	40	31	9	26	106
Total number infected samples	15	11	1	7	4
Total number of plants	695	548	164	461	1868
Total number infected plants	28	14	1	10	53
Total percent infected plants	4	3	1	2	3
Average percent infection in infected samples	11	7	7	8	9
Maximum percent infection in any sample	29	12	7	21	29
		1956			
Total number of samples	29	31	10	30	100
Total number infected samples	11	3	3	12	29
Total number of plants	745	799	237	767	2548
Total number infected plants	20	3	3	28	54
Total percent infected plants	3	Trace	1	4	2
Average percent infection in infected samples	7	4	5	9	8
Maximum percent infection in any sample	14	5	7	17	17

were cloudless. One group of seedlings was grown at 80° to 85° F under three layers of cheesecloth, the maximum light intensity on these plants being about 4000 foot candles. Another group was grown in an unshaded greenhouse at temperatures of 80° to 85°, and a third group in an unshaded greenhouse at temperatures ranging from 80° at night to as high as 110° during the day. These experiments also were repeated.

After determining the most favorable environment for symptom expression, farmers seed samples from the 1955 and 1956 Kansas barley crops were obtained from the State Seed Laboratory and were tested in the greenhouse to determine the distribution and prevalence of the disease in the State. The first test consisted of 57 samples of winter barley and was conducted in November and December, 1955. Twenty-five seeds from each sample were selected at random and were planted individually in 3-inch pots arranged far enough apart to eliminate contact between the young seedlings. These seedlings were grown at a constant temperature of 70° F in an unshaded greenhouse under bright sun and were observed daily for the appearance of symptoms. Infected plants were removed as symptoms were noted, and the remaining symptomless plants were cross inoculated to Pawnee wheat, Reno barley and Golden Giant sweet corn to detect possible symptomless carriers. The carborundum rubbing method of manual inoculation described by McKinney (3) was utilized. Forty-nine additional samples of winter barley were indexed on January and February, 1956, using the same technics.

The 1955 spring barley samples were indexed in the greenhouse in October and November, 1956, using a slightly different method. The samples were grown in greenhouse flats. Four rows were opened in each flat and 25 seeds from a seed sample were planted in each row. Thus, each flat contained four seed samples. Using this method, it was possible to test 120 samples simultaneously, using much less greenhouse space than was used in indexing the

Table 3. Summary of the occurrence of barley stripe mosaic virus in seed of Kansas spring barley varieties.

	Flynn	Beecher	Custer	Miscellaneous	Total
		1955			
Total number of samples	14	30	14	62	120
Total number infected samples	5	11	2	24	42
Total number of plants	251	578	285	1245	2359
Total number infected plants	7	20	2	58	87
Total percent infected plants	3	3	1	5	4
Average percent infection in infected samples	8	9	6	13	11
Maximum percent infection in any sample	12	16	7	33	33
		1956			
	Beecher	Custer	Otis	Miscellaneous	Total
Total number of samples	28	10	6	48	92
Total number infected samples	6	3	3	19	31
Total number of plants	642	236	135	1074	2087
Total number infected plants	7	3	3	25	38
Total percent infected plants	1	1	2	2	2
Average percent infection in infected samples	4	5	4	6	6
Maximum percent infection in any sample	8	5	5	24	24

winter barley samples. Greenhouse temperatures were maintained near 75° F, but temperatures of 80° and 90° occurred occasionally. Infected plants were removed as symptoms were noted, and the remaining symptomless plants were cross inoculated to Pawnee wheat, Reno barley and Golden Giant sweet corn, as before. Similarly, 100 1956 winter barley samples were indexed during November and December, 1956 and 92 1956 spring barley samples during February and March, 1957.

EXPERIMENTAL RESULTS

As mentioned previously, a spring barley seed sample which had a high percentage of infected seed was chosen for the environmental studies. When average light intensity was near 3000 foot candles but temperatures were maintained at 60° F, only 4 percent of the plants in one trial and 9 percent in a second trial developed symptoms. Using the same seed lot and light intensity 17 percent of the plants developed symptoms at 70°, and 13 and 16 percent in two trials developed symptoms at 80°. At 800 foot candles 15 percent developed symptoms in two trials at 70°, and 18 and 14 percent in two trials at 80° to 90°. At 4000 foot candles 20 percent developed symptoms at 80°. At 10,000 foot candles and 80° there was the highest percentage of plants showing symptoms (27 percent). When the temperatures climbed to 110° or slightly less at 10,000 foot candles, symptoms did not develop at all. The results of these experiments are presented in Table 1.

Some symptoms began to appear on the first emerging leaf as early as 6 days after planting when plants were grown at 70° and 80° F at high light intensities. At 60° initial symptoms were delayed usually until 15 days or more after planting.

The 206 farmers' winter barley seed samples tested were from widely separated counties in Kansas and were, as nearly as possible, a representative sample of the 1955 and 1956 Kansas barley crops. Of the 4416 plants grown from all of the winter barley samples, 73 were infected with the virus (Table 2). Infection in individual samples ranged from zero to as high as 29 percent. Cross inoculation to Pawnee wheat, Reno barley, and Golden Giant sweet corn revealed the presence of only one symptomless carrier in the winter barley samples grown

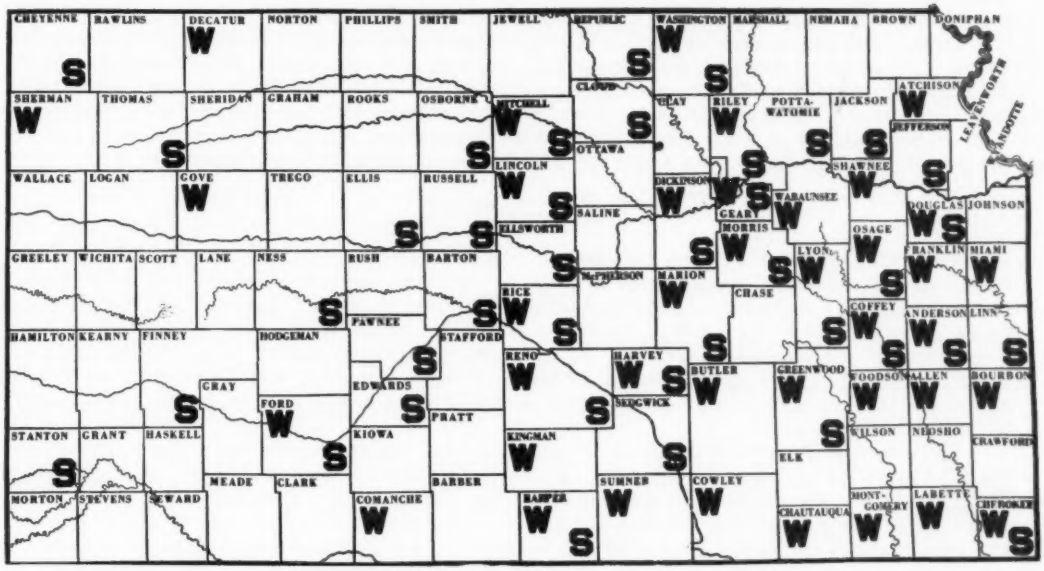

FIGURE 1. Kansas counties in which winter (W) and spring (S) barley seed was found to be infected with barley stripe mosaic virus in 1955 and 1956. This disease has also been reported in Clay, Marshall, McPherson, Saline and Smith counties (7).

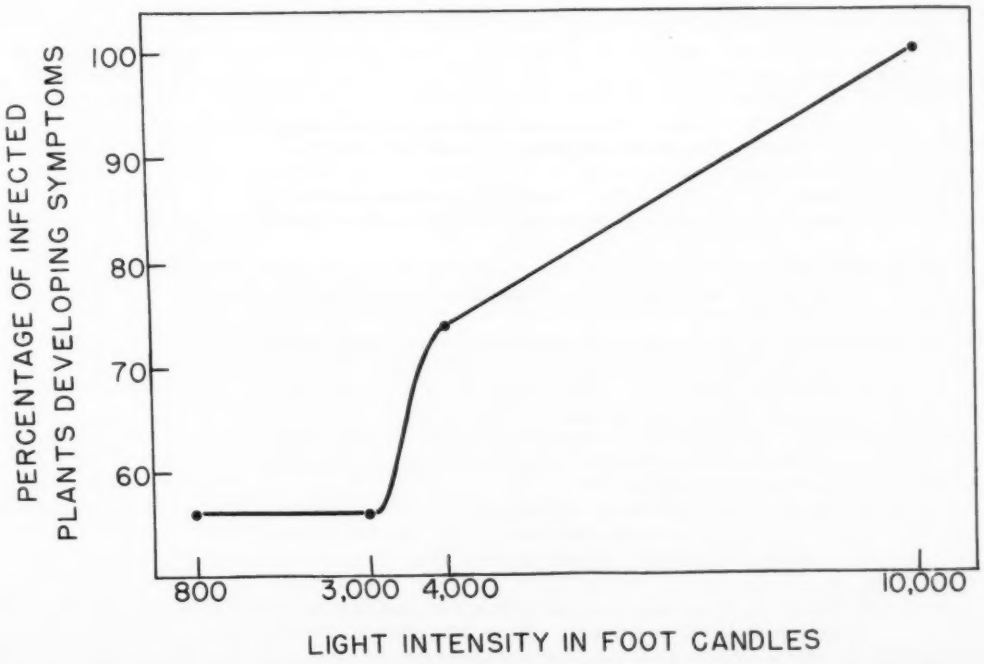

FIGURE 2. The effect of light on the development of barley stripe mosaic virus symptoms in seedling barley held at optimum temperatures (70° to 85° F).

under the optimum environmental conditions discussed previously. Figure 1 shows the distribution of infected seed samples in the 1955 and 1956 Kansas winter barley crops.

Of the 4446 plants grown from the spring barley seed samples, 125 were infected with the virus. These plants were from 73 of the 212 samples, and infection in these samples ranged from zero to as high as 33 percent. These data are summarized in Table 3, and the distribution is shown in Figure 1. Cross inoculation of the samples to Pawnee wheat, Reno barley, and Golden Giant sweet corn did not disclose the presence of symptomless carriers in any of the samples tested when grown under the optimum environmental conditions.

DISCUSSION

The data presented indicate that light and temperature exert a considerable influence on symptom expression. Temperatures of from 70° to 85° F and light intensities of near 10,000 foot candles were optimum for development of seedling symptoms in infected plants. Temperatures above and below the optimum level and low light intensities resulted in the more frequent occurrence of symptomless carriers. At optimum temperatures of 70° to 85°, light was the limiting factor in symptom expression. This is shown in Figure 2, which indicates the percentage of plants from a particular seed sample developing symptoms at light intensities between 800 and 10,000 foot candles.

The data also show that barley stripe mosaic virus was distributed throughout Kansas in the 1955 and 1956 spring and winter barley crops. An earlier report (7) gave a partial pattern of distribution in the State. Although the total infection in winter and spring barley was not high (2.6 percent), infection in individual samples was as high as 29 percent in winter barley and 33 percent in spring barley. Seed lots containing such high percentages of infected seeds would result in considerable reduction in yield. The low over-all infection percentage indicated a relatively small total loss in Kansas barley from this disease at the present time, unless there are strains which could not be detected by the technics utilized.

The method described for determining distribution and prevalence in Kansas barley could also be used as a seed certification technic, although probably a larger seed sample or perhaps 100 seeds should be used. This method might fail to detect seed lots containing very low percentages of infected seeds, but heavily infected seed lots could be detected easily. The infrequent occurrence of symptomless carriers under the favorable environmental conditions reported would make cross inoculation of the samples seldom necessary in a seed certification program.

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A VEIN-YELLOWING DISEASE OF CITRUS CAUSED BY A GRAFT-TRANSMISSIBLE VIRUS

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Abstract

A disease of limequat (lime x kumquat) trees characterized by yellowing of the veins of the leaves has been found in California. The causal agent is readily transmitted from citrus to citrus by grafting.

A virus disease has been found in limequat (lime x kumquat) trees in California distinguished by a vein-yellowing in citrus leaves which appears to be different from any of the previously described virus diseases of citrus. The petioles and veins of some of the leaves on affected trees appear slightly swollen and bright yellow. The yellowing is generally continuous along the petiole, midrib, and larger veins. It often extends into the tissue adjacent to the veins, giving them the appearance of being greatly enlarged. The discoloration is sometimes more pronounced on one side of the leaf than on the other but is visible on both sides. Smaller veins may not be uniformly affected and frequently there are yellowed areas separated by normal-appearing areas. In some cases the yellowing extends down from the petiole into the young twigs and branches to a distance of several inches. The symptoms are persistent and are commonly present in the old leaves. Figures 1, 2, and 3 illustrate the typical symptoms of the yellow-vein disease in the leaves of seedlings of various kinds of citrus.

Graft-transmission experiments showed that the virus could be readily transferred from limequat to West Indian lime seedlings (Figs. 1 and 2). Additional transmissions were made to other citrus species and varieties, and vein-yellowing symptoms have appeared in grafted seedlings of calamondin, Orlando tangelo, Troyer citrange, limequat (Fig. 3), lemon and kumquat. The earliest and most striking symptoms appeared in West Indian lime and calamondin seedlings. A high percentage of infection was obtained in all tests, and transmission was obtained in all cases where tissue grafting was successful. The time for appearance of symptoms after inoculation usually varied from 15 to 20 days in most cases, but in some plants symptoms were delayed as long as 6 weeks. In one experiment typical vein-yellowing symptoms appeared in the newly-developed leaves of West Indian lime seedlings 10 days after inoculation (Fig. 1).

Several attempts were made to transmit the virus from citrus to citrus by inoculation with juice expressed from diseased plants. Thus far none of the inoculated plants has developed the vein-yellowing symptoms.

In current surveys to determine the extent to which the disease is present in citrus plantings the vein-yellowing virus has been found in 3 out of 10 limequat trees distributed in two counties in California. Many citrus trees in addition to limequat have been examined, but there is no visual evidence thus far to indicate that the disease occurs in any other citrus varieties or species.

A bud-transmissible vein-yellowing disease was found in Eustis limequat at Indio, California in October, 1956 by J. B. Carpenter of the United States Date Field Station. The affected tree had several hundred leaves with vein-yellowing symptoms. Although this disease was transmitted to Mexican lime seedlings, bud inoculations in several varieties of limequats, citrangequats, and kumquats failed to reproduce the symptoms in those hosts during a period of more than 7 months. This is the only other known report of a similar disorder.

The nature and development of symptoms and the transmissibility indicate that the vein-yellowing disease of citrus is caused by a virus or viruses. At present it is not known whether the disease is the result of the multiple infection by two or more known viruses, or is due to a new strain of one of the known viruses or to a heretofore undescribed virus. The disease appears to be distinct from other recognized diseases of citrus. Experiments aimed at identifying the virus or viruses responsible for the disease and determining its causal relationship to other diseases of citrus, especially diseases of unknown cause, are now in progress.

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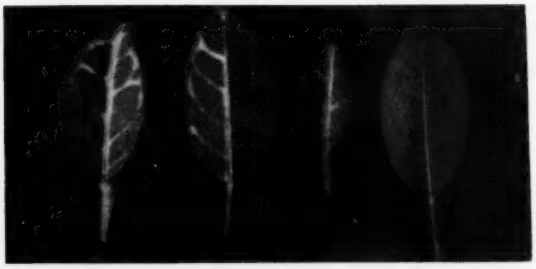

FIGURE 1. Leaves from West Indian lime seedlings with the yellow-vein disease, showing the yellowing of the veins 12 days after inoculation with tissue from diseased limequat (left) and leaf from normal plant (right).

FIGURE 2. Leaves from West Indian lime seedlings, showing yellow-vein symptoms 21 days after inoculation with tissue from diseased limequat (left) and normal leaf (right).

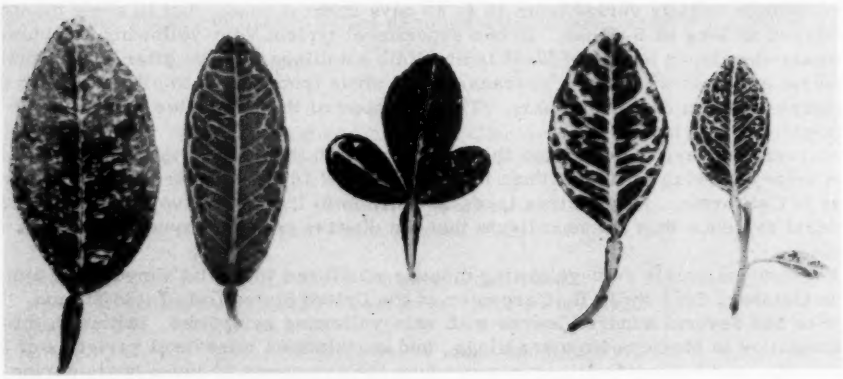

FIGURE 3. Symptoms of the yellow-vein disease on leaves of (from left to right) Orlando tangelo, limequat, Troyer citrange, calamondin, and West Indian lime seedlings. Note the yellowing of the attached branches on the leaves of calamondin and West Indian lime.

Particular emphasis will be placed on determining whether or not the disease is a threat to the culture of citrus.

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OBSERVATIONS ON ABNORMAL CITRUS ROOTSTOCK REACTIONS IN BRAZIL

T. J. Grant[1], S. Moreira[2], and A. S. Costa[3]

Summary

In a rootstock planting at Tiete symptoms consisting of pegs and an orange-yellow coloration occurred at the bud-unions of 8-year-old clonal Pera sweet orange tops on Florida Rough lemon and on trifoliate-orange rootstocks. Trees composed of the same Pera orange tops on sweet lime or on Rangpur lime showed no xyloporosis symptoms on these rootstocks. In this planting, similar but even more marked bud-union symptoms of indentation into the wood and cracking of the bark were observed on trees with Eureka lemon tops on trifoliate-orange rootstock.

In the Ribeirão Preto planting xyloporosis symptoms occurred on both sweet lime and Rangpur lime rootstocks with Baía navel orange tops. The inner bark and wood-pitting symptoms on Rangpur lime appeared first and were stronger at the ground level and developed upward toward the union.

Association of abnormal symptoms at the bud unions of sweet orange tops on Florida Rough lemon rootstock with xyloporosis and cachexia and the indicated differences in causal agents are discussed in relation to the observed rootstock reactions in Brazil.

INTRODUCTION

Since 1947 the Instituto Agronomico, Campinas, Brazil, and the United States Department of Agriculture have cooperated on investigations of the virus diseases of citrus, principally the disease caused by tristeza virus. In citrus areas where tristeza virus spreads rapidly tristeza-tolerant rootstocks must be used. With the utilization of new rootstocks in commercial plantings in Brazil the presence or absence of other viruses in old-line clonal sources of budwood has assumed importance. The tristeza-tolerant Rangpur lime was used as a rootstock in extensive commercial plantings and in experimental ones, but it soon showed symptoms of bark shelling and gum spots that Moreira (9) demonstrated to be caused by exocortis virus. Association of bark-shelling symptoms on Rangpur lime rootstock with exocortis virus was subsequently reported by Olson and Shull (10) in Texas and by Reitz and Knorr (12) in Florida. Since other diseases such as cachexia or xyloporosis could limit the commercial use of different varieties as rootstocks in Brazil, the need arose to determine whether cachexia and xyloporosis are caused by the same or different viruses.

Xyloporosis was first described by Reichert and Perlberger (11) as a disease of sweet orange on sweet lime rootstock. Cachexia was described by Childs (1) as a disease of Orlando tangelo and later (2) he pointed out the similarity of bark symptoms of cachexia to those of xyloporosis. Grimm et al. (6) described a bud-union abnormaility of sweet orange on Florida Rough lemon rootstock. Upon removal of the bark at the bud union they found a dotted to continuous line of orange-yellow discoloration on the inner bark surface associated with rounded protrusions and pegs that extended from the bark into corresponding depressions in the wood. They suggested that the bud-union abnormality might be due either to genetic incompatibility or to a virus such as that causing cachexia. Marked symptoms of the bud-union disorder were not observed in all sweet orange trees on Rough lemon, in any trees having grapefruit tops and Rough lemon rootstock, or in any sweet orange trees on sour orange rootstocks. More extensive observations on the reactions of these scion-rootstock combinations were reported by Childs et al. (4), together with data on association of the abnormal bud-union symptoms of sweet orange on Florida Rough lemon rootstock with cachexia, as expressed on Orlando tangelo test plants in the budwood certification nursery of the Florida State Plant Board. On the basis of similarity of bark symptoms and transmission tests by Childs (3), cachexia and xyloporosis were considered to be caused by the same virus and the name xyloporosis was adopted (4).

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Table 1. Disease symptoms on rootstocks or at the bud unions of different citrus varieties in the experimental planting at Tietê, Brazil[a].

Rootstock variety or kind	Hamlin sweet orange top	Baianinha[b] sweet orange top	Maracaná sweet orange top	Pera[c] sweet orange top	Mexerica tangerine top	Eureka lemon top
Trifoliate-orange	Bark shelling Exocortis[d]	Bark shelling Exocortis	Bark shelling Exocortis	Bud-union Pegs + orange-yellow ring	0	Bud-union Pegs + orange-yellow ring Bark cracking
Rangpur lime	Bark shelling Gum spots Exocortis	Bark shelling Gum spots Exocortis	Bark shelling Gum spots Exocortis	0	0	0
Sweet lime[e]	0	0	0	0	0	0
Florida Rough lemon	0	Very few small pegs on bark	Very few small pegs on bark	Bud-union Pegs + orange-yellow ring	-	0
Brazilian Rough lemon	0	0	-	0	-	0
Caipira orange	0	0	0	0	0	0
Pera orange	0	0	0	0	0	0
Cravo tangerine	0	0	-	0	-	0
Shaddock	-	-	-	-	-	0
Cuban shaddock	-	-	-	-	-	0
Sour orange	-	-	-	-	-	0
Sampson tangelo	-	0	-	-	0	-
Cleopatra mandarin	-	0	-	-	0	-

[a] Each rootstock and scion combination was represented by three trees in each plot with three replications planted December 1948. Each scion variety originated from buds from a single tree.

[b] Baianinha navel orange also has psorosis virus as indicated by leaf symptoms. No psorosis symptoms have been found on the other varieties or on the parent trees.

[c] The Pera sweet orange was from an old established clone at the Limeira Citrus Experiment Station and by tests considered free of psorosis and exocortis viruses and free of xyloporosis virus as measured by tree development on sweet lime rootstock. Tristeza virus is considered as present in all sweet orange varieties and in the tangerine.

[d] Typical bark shelling on rootstock indicative of presence of exocortis virus.

[e] Sweet lime (Lima da Persia) shown to be a host for xyloporosis (7).

Olson and Shull (10) in Texas made transmission tests from a Marsh white grapefruit tree on a Rangpur lime rootstock that showed xyloporosis symptoms and obtained symptoms of xyloporosis on sweet lime and symptoms of xyloporosis (cachexia) on tangelos. Olson and Shull employed the term xyloporosis as synonymous with cachexia but Olson expressed the opinion that more definite evidence was needed to prove that the causal agents of these two diseases were the same.

These findings, supplemented by observations on various citrus scion-rootstock reactions under field conditions in Brazil and in Florida, stimulated us to examine trees in the rootstock plots in Brazil to determine whether additional data might be obtained to substantiate the identity of or to differentiate xyloporosis and cachexia and their causal agents.

OBSERVATIONS IN CITRUS PLANTINGS

Tietê Rootstock Planting

The 8-year-old citrus planting at Tietê is on the grounds of a sub-station of the Instituto Agronomico of Campinas. The observations are reported in Table 1. The value of the comparative readings is greatly enhanced by the fact that all buds used for each variety top came from single tree sources.

The occurrence of bark-shelling symptoms typical of exocortis virus on trifoliate-orange rootstock and of similar but less prominent symptoms on Rangpur lime rootstock with Hamlin, Baianinha and Maracanã sweet orange tops suggests an identical cause of these disease conditions as noted by Moreira (9). At the same time the lack of any symptoms on sweet lime (Lima da Persia) rootstock with these same tops showed their freedom from xyloporosis infection (7). The presence of psorosis virus in Baianinha and its absence in Hamlin and Maracanã oranges did not affect the degree of bark-shelling symptoms of exocortis on the trifoliate-orange or Rangpur lime rootstocks. The exocortis symptoms began near the ground line and extended upward on the stem, in some cases reaching the bud union 12 to 20 inches above.

The reactions of rootstocks with Pera sweet orange tops were in distinct contrast to those with the other three sweet orange tops tested. The Pera orange top was free of exocortis virus as shown by the absence of bark shelling on trifoliate-orange and Rangpur lime rootstocks. It was free of xyloporosis as indicated by the absence of symptoms on sweet lime rootstock. That it was free of psorosis virus was indicated by extensive observations on this old clone selection over a period of years at the Limeira Citrus Station. In fact this clonal selection of Pera orange was thought to be free of all viruses except tristeza virus, which is widespread and considered present in all sweet orange scions, until the abnormal bud-union symptoms of pegs and an orange-yellow ring (Table 1) were found when this Pera orange top was on Florida Rough lemon and trifoliate-orange rootstocks.

The abnormal bud-union symptoms on Pera orange on Florida Rough lemon rootstock occurred on all 12 trees in the 4 replicated blocks and were typical of the bud-union abnormality of Rough lemon rootstock with sweet orange scions previously described and associated with cachexia (4, 6). The occurrence of similar abnormal symptoms of pegs, yellow coloration, and some bark cracking at the bud union of Pera orange tops on trifoliate-orange rootstock suggests that this condition might also be due to the same or a similar causal agent. The symptoms observed at Tietê, Brazil, were confined to the bud unions and were not the rugose wood symptoms observed on Rough lemon rootstock or the conoid pit symptoms observed on trifoliate-orange rootstock by DuCharme and Knorr (5) in Argentina.

The similar but even more striking bud-union symptoms of indentation in the wood and bark cracking on trifoliate-orange rootstock with Eureka lemon tops were essentially the same as the symptoms described by Weathers et al. (13) for old-line nucellar Eureka lemon tops on trifoliate-orange and Troyer citrange rootstocks.

The presence of bud-union symptoms of pegs and an orange-yellow ring on the Florida Rough lemon and their absence on Brazilian Rough lemon with the same Pera orange top emphasized the differences in reaction of these Rough lemons. Moreira (8) described other characteristic differences of these Rough lemon varieties.

Ribeirão Preto Rootstock Planting

The citrus planting at this sub-station was made 20 years ago. Each scion-rootstock combination was planted in a 10-tree row in each of four blocks. Many of the trees are now

missing because of the non-tolerance of the rootstocks such as sour orange to tristeza virus.

The importance of observations in this older planting rests on the fact that some of the Baía navel orange tops were known to be infected with xyloporosis virus as indicated by symptoms on some trees with sweet lime (Lima da Persia) rootstock. It was from this planting that A. S. Costa obtained bark samples that were chemically preserved and sent to Childs (2) for comparison with cachexia on Orlando tangelo.

Observations in this planting showed that many of the surviving Baía navel orange trees were on Rangpur lime rootstock. Many of these showed some decline. Examination of the rootstocks showed no external symptoms of exocortis infection (9). Upon removal of bark patches from the trees in two rows, 12 of 20 trees showed bark pegs and corresponding depressions in the Rangpur lime wood and orange-yellow to dark-brown gum deposits in the bark. These symptoms were present to some degree at the unions but were more pronounced lower on the rootstock. These symptoms might easily be ascribed to either cachexia or xyloporosis. There was no proof of the presence or absence of cachexia, but there was definite evidence of the presence of xyloporosis as measured by the reaction on sweet lime rootstock in this planting. Thus, it seems reasonable to associate the symptoms on Rangpur lime with xyloporosis virus.

The absence of symptoms on some trees with Rangpur lime and sweet lime rootstocks suggests that the buds for this early experiment were taken from more than one tree. No detailed record of bud source was available.

Commercial Citrus Plantings

The majority of the citrus plantings in São Paulo are less than 10 years old because from 1937 to 1947 nearly 8 million orange trees on sour orange rootstock were killed by tristeza virus. Some losses of sweet orange trees on sweet lime rootstocks were due to a combination of tristeza and xyloporosis. Most of the surviving 20- to 30-year-old citrus trees are on Caipira sweet orange rootstock, which is tolerant to both tristeza and xyloporosis. In a few older plantings the sweet orange trees are growing on Rangpur lime rootstock.

In one 24-year-old planting of Barão sweet orange on Rangpur lime rootstock at Bebedouro some trees showed stunting and decline symptoms but no definite external symptoms of exocortis. Bark patches were removed and some showed characteristic xyloporosis symptoms. In an adjacent 4- to 5-year-old planting with the same Barão sweet orange top on Rangpur lime rootstock, about 30 percent of the young trees were smaller than the others. Examination of these revealed typical xyloporosis bark symptoms in the rootstock. Again, it was noted that the symptoms were strong at the root crowns and in many cases they had not reached the bud union 12 to 20 inches above.

These observations did not form a basis for distinction between xyloporosis and cachexia but did indicate that the causal agent, uncontaminated by exocortis virus, was causing some damage in the older planting and appeared likely to cause even more damage in the younger grove.

Limeira Rootstock Plots

At the Limeira Citrus Experiment Station observations were made in two 20-year-old plantings (8). The first had plots of 9 trees of each rootstock-scion combination in each of 4 blocks and the second, plots of 4 trees of each rootstock-scion combination in each of 4 blocks. Pera sweet orange tops in these plots were from the same single-tree bud source as those in the Tietê planting. None of the sweet lime or Rangpur lime rootstocks showed any xyloporosis symptoms. Likewise, there were no definite symptoms of pegs or discoloration at the bud unions of the Pera orange tops on Brazilian Rough lemon rootstock. Florida Rough lemon was not included as a rootstock in these plots. There were, however, 4 plots of 4 trees each of 20-year-old Pera orange tops on trifoliate-orange rootstock. All of these showed some degree of indentation at the bud union and an orange-yellow discoloration. The symptoms were similar to those at Tietê but were not as marked.

DISCUSSION

Because of the similarity of bark symptoms of cachexia on Orlando tangelo to that of xyloporosis on sweet lime rootstock (2), and because of transmission tests (3), cachexia and xyloporosis were considered to be the same and the name xyloporosis was employed. For purpose

of this discussion the term cachexia will be limited to the described disease (1) on Orlando tangelo and xyloporosis to the described disease (11) on sweet lime. In Florida abnormal pegs and orange-yellow coloration at the bud-union of sweet orange on Florida Rough lemon rootstock have been associated with the presence of cachexia as expressed on Orlando tangelo test plants (4, 6). It was considered that these symptoms at the bud-union of sweet orange tops on Rough lemon rootstock might be an indication of presence of the virus that causes cachexia.

The observations at Tietê, Brazil, show that something is wrong in assuming a relation of cachexia and xyloporosis to abnormal bud-union symptoms of pegs and an orange-yellow ring on Florida Rough lemon rootstock. Typical bud-union symptoms were produced on Florida Rough lemon rootstock with Pera sweet orange top, but the sweet lime and Rangpur lime rootstocks with the same tops showed no symptoms of xyloporosis. If one accepts the bud-union symptoms of pegs and an orange-yellow ring on Florida Rough lemon rootstock as indicative of the presence of cachexia virus then it follows, because of the lack of xyloporosis symptoms on sweet lime, that cachexia and xyloporosis are caused by two distinct viruses. If, on the other hand, the causal agent of xyloporosis is the same as that of cachexia, then the bud-union symptoms on Florida Rough lemon at Tietê would have to be interpreted as due to some other cause such as incompatibility or another virus.

Observations in Brazil, however, indicated that tristeza virus alone or in combination with exocortis or exocortis and psorosis viruses had not caused marked bud-union symptoms on the Florida Rough lemon rootstock of the trees observed.

The cause of the marked bud-union symptoms on Florida Rough lemon rootstock with the Pera orange top is not known. The lack of symptoms on trees consisting of Pera orange top on sweet lime (Lima da Persia), which is sensitive to xyloporosis, would indicate that this virus was not involved in this instance. Tests to determine the presence or absence of cachexia virus by bud transmission to Orlando tangelo and tests of clonal and nucellar seedling Pera orange tops on several different rootstocks are underway.

The finding at Tietê of similar bud-union symptoms of pegs and an orange-yellow discoloration on trifoliate-orange rootstock with Pera orange tops might indicate that whatever factor is present in the Pera orange top affects both trifoliate-orange and Florida Rough lemon.

The similar but more extreme bud-union symptoms of indentation in the wood and bark cracking on trees consisting of Eureka lemon tops on trifoliate-orange rootstock would at first appear to be due to a similar but even stronger factor than that present in the Pera orange top on this rootstock. However, the complete lack of symptoms when the Eureka lemon top was on Florida Rough lemon rootstock would indicate that the Eureka lemon was not carrying or producing one of the factors present in the Pera orange top.

Weathers et al. (13) in California showed that nucellar as well as old-line clones of Eureka lemon as tops on Troyer citrange and on trifoliate-orange can cause bud-union symptoms. Their described bud-union symptoms when Eureka lemon was on Troyer citrange and trifoliate-orange rootstocks are similar to, if not identical with, the bud-union symptoms on trees consisting of Eureka lemon tops on trifoliate-orange rootstock that we observed at Tietê. Weathers et al. suggested that this disorder as observed in California may be caused by a virus or by a form of incompatibility between the Eureka lemon top and the Troyer citrange.

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UNITED STATES DEPARTMENT OF AGRICULTURE, AGRICULTURAL RESEARCH SERVICE, ORLANDO, FLORIDA; AND INSTITUTO AGRONOMICO, CAMPINAS, SÃO PAULO, BRAZIL

THE ARKANSAS STRAWBERRY CERTIFICATION PROGRAM[1]

J. P. Fulton and Carter Seymour[2]

Summary

The operation of a strawberry certification program in Arkansas is outlined. Indexing of plants to eliminate virus-diseased clones is done in greenhouses during the winter. Clones essentially free of plant-borne pathogens are increased for one season in screenhouses. The first field increase from screenhouse-grown plants is produced by the Arkansas Agricultural Experiment Station and sold to growers as Purple Tag plants. Commercial growers produce the next two increases which are certified as Blue Tag plants and Green Tag plants. The aim of the program is to make available to growers plants which are free of plant-borne pathogens.

A program for the certification of strawberry plants has been developed by the Arkansas Agricultural Experiment Station in cooperation with the Arkansas State Plant Board. The object is to make available to growers plants which are free of the major strawberry pathogens. In certain phases of the program emphasis is placed on virus diseases. Other plant-borne pathogens receive due consideration and practices are maintained to increase clones essentially free of disease producing agents. Plants produced under this certification program were first made available to commercial growers in the spring of 1955. In the spring of 1957 43 percent of the strawberry plants offered for sale by strawberry plant producers in Arkansas were certified under this program. This paper outlines procedures followed at all levels in the production of Arkansas Certified Strawberry Plants.

INDEXING STRAWBERRY CLONES

Clones of the various strawberry varieties essentially free of disease have been obtained from several sources. In some cases clones were introduced into the program after indexing plants from local sources. Many of the varieties which are not fruited commercially in Arkansas have been obtained from the Agricultural Research Service Station of the United States Department of Agriculture at Beltsville, Maryland. The variety Florida 90 was supplied by Dr. A. N. Brooks of the Strawberry Field Laboratory, Lakeland, Florida.

In the fall of the year two to four plants of each variety used in the program are set in crocks of sand in the greenhouse. These plants are from an increase produced in screenhouses during the summer months. Plants are watered with a complete nutrient solution and exposed to 3 to 4 hours additional light by artificial illumination. Experience indicates that this method of handling facilitates most rapid runner production. Runner plants are pinned down in pots of sterilized soil. As each "daughter plant" is established in soil the runner is severed and the pot is placed in a different section of the greenhouse. These potted plants continue to receive additional light and produce runners. Additional plants are collected until a sufficient supply is obtained to establish the screenhouse planting of each variety.

During the winter months the mother plants growing in the crocks of sand are indexed for the presence of viruses. Several different sources of the wild strawberry, Fragaria vesca L., have been used as virus indicators. These include two sources of the East Malling Clone (11), one carrying strawberry latent virus, strain A (code BEM) (4), one apparently free of this virus (code EMK); the runnerless Alpine seedling (code AS); and the clone (code BE-4) of a hybrid from a cross between EMK and AS (7). It may not be necessary to use all of these indicators in each case. Experience has demonstrated, however, that dependence upon a single indicator plant often leads to erroneous results regarding the virus content of the plant being indexed. In some cases indicator plants from the above types containing known viruses are used. This is particularly true concerning tests for the strawberry latent virus where use is made of in-

[1] Published with approval of the Director of the Arkansas Agricultural Experiment Station, Fayetteville, Arkansas, and Chief Inspector of the State Plant Board, Little Rock, Arkansas.

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dicator plants containing the mild mottle virus (4).

Several types of grafts have been used in indexing. The excised leaf grafting technique as described by Bringhurst and Voth (1) is helpful for preliminary screening of plants shortly after they have been placed in the greenhouse. This method has the advantage of not contaminating the plant being indexed and in saving greenhouse space. Later, after no additonal "daughter plants" are to be collected from a "mother plant", use is made of runner grafts or runner to petiole grafts (11).

In indexing each "mother plant" at least 10 and often 25 or more grafts are made. If obvious virus symptoms appear in any of the indicator plants, the "mother plant" from which the grafted tissue was obtained is discarded. With the indicator plants listed above, mild mottle (15) (virus 1 of Prentice and Harris (13)) and virus C (12, 15) (type 2 virus of Demaree and Marcus (3)) are readily indicated. It is recognized that several strawberry viruses produce very mild or no symptoms at all in the indicator plants. Strawberry latent virus (4) is of this type but it is by no means the only virus which is not adequately indicated by the indexing procedure outlined above. At present, to indicate strawberry latent virus, use is made of the synergistic reactions between that virus and mild mottle (4) or between that virus and vein banding virus (5). As techniques are developed for indicating viruses which are not now indicated efficiently, they will be introduced into the program.

Plant-borne pathogens, other than the viruses, are controlled by increasing only clones which have proven to be free of these pathogens and avoiding all possibilities of contaminating these clones. In the past plants have received a hot water treatment as developed by Staniland (17) of 115° F for 10 minutes to eliminate the possible presence of the summer dwarf nematode (16). Although this practice may not be necessary, since disease-free lines are used, it is a valuable precautionary measure.

SCREENHOUSE OPERATION

Two types of screenhouses are currently in operation (Fig. 1). One type is a rectangular unit measuring 22 feet by 12 feet which is closed by a double door entry (Fig. 2). The second type is a small unit 8 feet by 12 feet of an inverted V shape (Fig. 3). The small units were constructed for isolation of single varieties. The screen-covered frame of this unit can be removed for ease in thorough fumigation of the soil (Fig. 4). The door of the small unit is locked during the growing season and entered only when necessary. All screenhouses are covered with Saran plastic screen of a 32 by 32 mesh.

Prior to the growing season soil in the screenhouses is fumigated with methyl bromide at the rate of 4 pounds per 100 square feet. Plants from the greenhouse stocks mentioned above are set in the screenhouses during April. Plants and soil in the screenhouses are thoroughly sprayed with demeton to control insects. In five years' operation no evidence has been obtained of any virus being introduced into screenhouse plants by insects. No parasitic nematodes have been recorded as being present on plants produced in the screenhouses.

FIELD PRODUCTION OF PURPLE TAG PLANTS

The first year's field increase of strawberry plants from the screenhouse plants is produced by the Arkansas Agricultural Experiment Station at one or more of its substations. No other strawberry plants are under cultivation at the stations used in this program and the stations are isolated from commercial strawberry production. The soil for this field planting has not previously been in strawberries and is treated with methyl bromide under plastic covers at the rate of 1 pound per 100 square feet. This treatment is usually made in the late fall or early winter. Plants are dug from the screenhouses and set in the field in March. These field plantings are subjected to the same field inspections by inspectors of the Arkansas State Plant Board as are made of Blue Tag plantings outlined later. No routine insecticide applications are made. Plants from these fields have been indexed since the program has been operating. As yet no evidence has been recorded of a virus, other than a very few cases of the aster yellows virus (9), having been introduced into these plantings by insects. The plants are sold to commercial growers as Arkansas Certified Purple Tag plants.

FIELD PRODUCTION OF BLUE TAG PLANTS

Certified Blue Tag strawberry plants are produced by a limited number of commercial growers who can meet the requirements for this grade. These requirements are: 1 -- Plants

FIGURE 1. View of some of the screenhouses currently in operation. Greenhouse used in indexing is in the background.

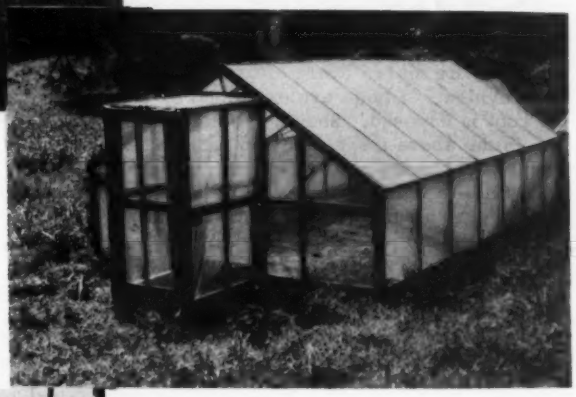

FIGURE 2. One of the large screenhouses.

FIGURE 3. One of the small screenhouses.

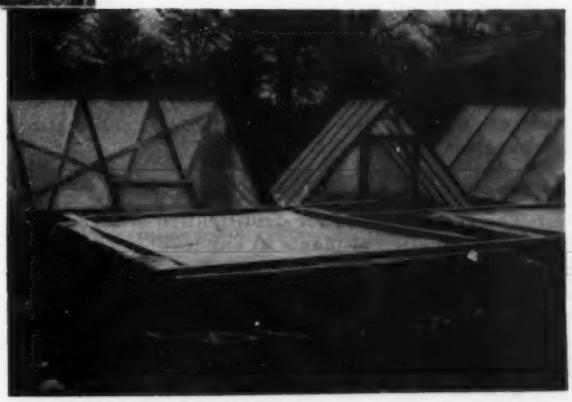

FIGURE 4. Small screenhouses removed from bases to facilitate thorough fumigation of the soil.

used must be Certified Purple Tag stock. 2 -- Plants must be located so that at least 1 mile separates them from any other strawberry planting. 3 -- Ground must not have been used to grow strawberries within the past 10 years. 4 -- Ground must be at least 1 mile from any area known to be infested with the red stele pathogen (Phytophthora fragariae).

Blue Tag plantings are given the following inspections by inspectors of the Arkansas State Plant Board: 1 -- June inspection for diseases which are likely to appear at this time of year, such as June yellows (genetic) and spring dwarf (Aphelenchoides). 2 -- September inspection for diseases appearing at this time of year, such as leaf spot (Mycosphaerella), summer dwarf (Aphelenchoides), June yellows, and root-knot nematode (Meloidogyne). This inspection is made between September 1 and October 31. Inspectors dig and examine not less than 100 plants per acre, taking plants from not less than 15 places per acre, well distributed. Plants may be dug and shipped at any time following fall inspection up to March 1, after which a red stele inspection is made before new certification tags are issued. 3 -- In making the red stele inspection, the inspector digs and examines not less than 100 plants per acre, taking plants from a number of places. The location of places for digging plants is at the discretion of the inspector. He makes every effort to dig plants in areas where red stele disease is most likely to be evident. Plants are examined by slicing several roots on each plant so that the stele of the root is exposed. Should even one plant show red stele, the entire field is disqualified.

In the above inspections no tolerance of red stele disease, root-knot nematode, or noxious weeds (field bindweed, wild onion, nut grass, and Johnson grass) is allowed. Current regulations allow a 0.1% tolerance of spring dwarf, summer dwarf, and June yellows; a 0.5% tolerance of crown borer, and a trace of leaf spot. Even though the regulations currently allow a tolerance of spring dwarf and summer dwarf, these diseases have not been observed in Blue Tag and Green Tag plantings.

Blue Tag producers sign a contract with the Arkansas Agricultural Experiment Station which obligates them, in addition to the purchase price of Purple Tag plants, to pay a specified fee to the Experiment Station for every Blue Tag plant sold. Funds received from these royalties are used in support of indexing, screenhouse operation, and Purple Tag production.

FIELD PRODUCTION OF GREEN TAG PLANTS

Certified Green Tag strawberry plants are produced by growers who meet the following requirements: 1 -- Plants must be certified Blue Tag Grade. 2 -- Plantings must be located at least 300 yards from any other strawberry planting. 3 -- Ground must not have been used to grow strawberries within the past five years. 4 -- Ground must be located at least 1 mile from any area known to be infested with the red stele pathogen. Field inspections are the same as those required in Blue Tag production.

RESEARCH IN SUPPORT OF STRAWBERRY CERTIFICATION

Active research on strawberry diseases and particularly problems related to the certification program is being conducted by the Arkansas Agricultural Experiment Station. Such investigations are essential in development and growth of this program. Results of some of this work have been published (6, 7, 8, 9, 10, 14, 15, 16). Further studies are in progress on the separating and characterizing of strawberry viruses. As indicated, methods of indexing are inadequate for certain viruses. Strawberry plants produced under the certification program are now planted throughout Arkansas in a variety of situations. This presents an excellent opportunity for the study of the reinfestation and spread of viruses into these stocks. Results of such studies are necessary in formulating isolation requirements. It is anticipated that research results will contribute to modifications and refinements of the program currently in operation.

DISCUSSION

The requirements for certification of strawberry plants outlined here do not provide for the recertification of plants at any level. The different grades available to growers (Purple Tag, Blue Tag, Green Tag) represent different periods of time from a carefully indexed plant. Although indexing of plants from fields of these grades is done from an experimental standpoint, it is not a required practice for certification. The large amount of greenhouse space required for adequate sampling of field populations makes such a practice impractical in the present program.

Neither soil treatment nor use of insecticides is required by growers of Blue Tag or Green

Tag plants. The nematode problems are being investigated carefully. To date, root knot has not been a problem in Blue Tag and Green Tag fields. No tolerance of root knot is allowed in either grade of plants and growers have experienced no trouble in meeting this requirement. Very low populations of Pratylenchus coffeae (Zimm.) Sher & Allen or P. penetrans (Cobb) Sher & Allen have been encountered in a few Blue Tag and Green Tag plantings. Soil treatment studies have, as yet, revealed no method which would eliminate these low populations. The program relies on isolation rather than the use of insecticides to prevent virus reinfestation. Studies in progress should indicate whether greater or lesser isolation will be required in the future. Insecticides will be required only when it can be shown that their use is necessary and will prevent virus reinfestation.

It should not be implied that this outline of Arkansas Strawberry Certification is presented as a model program nor is it suggested that the same procedures be followed by other states or groups developing regulations for strawberry plant production. Various areas of this country may well require different procedures and, thus, promoting a uniformity of regulations may hinder desirable programs. It is believed, however, that the various areas may profit by developing a uniformity of attitude regarding the production of plants free of serious plant-borne pathogens. There has been a blind acceptance by the public of the term "virus-free" as meaning free of disease producing agents. Thus, if an agency allows the sale of "virus-free" plants produced under a program which ignores certain of the virus problems, or inadequately handles the red stele or root knot diseases, it does an injustice to those states or agencies attempting to develop programs on a firm, scientific basis. This paper has outlined the program as developed in Arkanas to present the attitude of commercial producers, regulatory personnel and research staff concerned with developing and maintaining the program in this State.

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ARKANSAS AGRICULTURAL EXPERIMENT STATION AND ARKANSAS STATE PLANT BOARD

OBSERVATIONS ON THE EFFECT OF FUNGICIDES ON GRAY MOLD AND LEAFSPOT AND ON THE CHEMICAL COMPOSITION OF STRAWBERRY PLANT TISSUES

R. S. Cox and J. P. Winfree[1]

Abstract

In field trials, zineb and nabam provided best control of leafspot, dichlone and Dyrene were almost as good, thiram, was least effective. Nabam-zinc sulfate and zineb appeared to increase Botrytis gray mold. Dichlone provided the best control of gray mold while thiram and Dyrene were almost as effective. Nabam-zinc sulfate caused moderate leaf chlorosis, zineb caused slight chlorosis. Neither material noticeably stunted the plants. Dichlone caused severe stunting but no noticeable leaf damage. Dyrene caused a slight bronzing of the leaf and some stunting. There were no differences between treatments in respect to total calcium, magnesium, iron, potassium, or in nitrogen fractions as determined by chemical analyses. There was significantly more calcium and magnesium in the older than the younger leaves. Zinc accumulation was as much as ten times greater in plants sprayed with nabam-zinc sulfate or zineb than in untreated plants or those sprayed with other fungicides.

Recently (1) evidence was presented to the effect that through repeated application of certain dithiocarbamate fungicides (nabam, zineb, maneb) the severity of gray mold of tomato was increased. Several possible hypotheses for explaining this were suggested. One of the most intriguing of these was that increased susceptibility might result from an interaction of the applied chemical and the host tissues. Work was undertaken to determine if this phenomenon occurred in the case of gray mold, caused by Botrytis cinerea Pers., on strawberries.

DISEASE CONTROL

Materials and Methods: Florida-90 was the variety used. Plants were set out October 6, 1956. Experimental design consisted of single 50-foot rows (approximately 40 plants to the row) in randomized blocks. Materials were applied with an experimental spray rig (2) delivering 300 p. s. i.. Nozzle arrangement consisted of two overhead until December 19. After that one was employed overhead and one was dropped to either side of the row. The Meyer's Jumbo nozzle with #3 orifice was used. Gallonage output per acre was 56 through December 14 and 83 thereafter. A total of 30 sprays were applied on an approximate 5-day schedule during the test period (November 7, 1956 - April 3, 1957). Harvest operations began on January 8 and continued through April 5. Yield of healthy fruit was recorded directly by weight. The number of diseased fruit was recorded and later converted to weight by using for the conversion factor the average weight of the healthy fruit. Disease and vigor ratings were on a 0 to 11 basis, where 11 equals the maximum.

Experimental Results: Unfortunately for the Botrytis work, leafspot, caused by Mycosphaerella fragariae (Schw.) Lind., became epiphytotic, and undoubtedly affected development of gray mold in the check plots (Table 1). Zineb (Parzate powder) and nabam (Parzate liquid)-zinc sulfate provided best control of leafspot while thiram (Thylate) was least effective.

Interpretation of the effect of the materials on gray mold is further confounded by the fact that two of the materials, Phygon and Dyrene, caused injury to such an extent that the plants were noticeably stunted (note under "vigor", Table 1). Nabam-zinc sulfate and zineb, the latter to a much lesser extent, caused injury in the form of a chlorosis, but this did not appear to affect plant vigor.

In spite of the additional variables (leafspot and phytotoxicity), several interesting observations concerning gray mold are apparent from Table 1. It is evident that there was significantly more gray mold-diseased fruit in the zineb and nabam-zinc sulfate plots than in any of the others. It is also interesting that the amount of molded fruit from the check plots was intermediate be-

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Table 1. Effect of several fungicides on the development of Mycosphaerella leafspot, on Botrytis gray mold on fruit, and on vigor and yield of strawberry plants.

Materials [a]	Mycosphaerella leafspot			Vigor	Yield		
	2/13/57	3/11/57	3/19/57	3/19/57	Healthy (lbs.)	Diseased (Gray mold) (lbs.)	percent
Check	4.6	6.0	6.0	7.2 [b]	12	12	50
Parzate (zineb)	1.1	1.0	1.0	11.0	12	19	58
Parzate (nabam) plus Zn sulfate	1.4	0.8	1.0	10.0	14	17	55
Thylate	2.2	2.2	1.9	9.9	16	13	44
Phygon	2.4	1.5	1.0	6.5 [c]	14	9	40
Dyrene	2.0	1.2	1.2	8.7 [c]	16	13	47
L.S.D. .05	---	---	---		N.S.	3	11
.01	---	---	---		N.S.	4	N.S.

[a] Amount of commercial formulation in 100 gallons and percent active ingredient as follows: Parzate (2/100), 65% zineb; Parzate (2 qts.), 19% nabam; Thylate (1 1/2 lbs.) 65% thiram; Phygon (3/4 lbs.), 50% dichlone; Dyrene (2 lbs.), 50% 2, 4 dichloro-6-(o-chloroanilino) triazine.

[b] Low vigor rating the result of defoliation by Mycosphaerella fragariae.

[c] Low vigor rating due to phytotoxicity.

tween these two groups when figured on a percentage basis. This agrees with previous findings on tomatoes (1). The percentage rather than the total number of diseased fruit probably reflects more accurately the effect of the chemicals on gray mold development. Total yield is obviously affected by plant vigor as well as by gray mold, and plant vigor is a direct reflection of phytotoxicity and leafspot severity. The microclimate would be more favorable for gray mold in the case of the more luxuriant plant growth.

It is also well to keep in mind that the yield data represent 14 pickings. Many of these were made during periods when gray mold was at a low level, thus minimizing the effect of the chemicals.

CHEMICAL COMPOSITION

Materials and Methods: Leaves from all plots except those sprayed with Dyrene were sampled twice. Two whole leaves (leaflets and petioles) were taken from each plant of the 20 plots. Older leaves were selected on April 16, 1957 (lower one-third of the plant). Younger leaves (upper one-third of the plant) were selected April 30. The leaves were washed in dilute Alconox solution, rinsed thoroughly in distilled water, and dried at 85° to 90° C in a forced-draft oven. They were then ground in a Wiley mill with a stainless steel screen.

All nitrogen determinations followed A. O. A. C. Kjeldahl procedures (5). Protein-N was determined following 24-hour extraction in a soxhlet with 75 percent ethyl alcohol. Total calcium, magnesium, and potassium were determined on the Beckman Model DU spectrophotometer with flame photometer attachment (hydrogen-oxygen) following wet digestion with a nitric-perchloric acid mexture. The zinc-determination was according to the mixed-color dithizone method of Sandel (6); density readings were accomplished with a Bausch and Lomb Spectronic 20 set at 620 mμ. Total iron was determined, following wet digestion, by the o-phenanthroline method (6).

Table 2. Effect of several fungicides on the chemical composition of strawberry leaves.

Materials	N-Fractions			K	Ca	Mg	Fe
	Total-N (%)	Protein-N (%)	NH_4-N (ppm)	(parts per million)			
			(OLDER LEAVES)				
Check	3.36	3.09	28	218	112	49	128
Parzate (zineb)	3.25	3.01	24	221	112	50	123
Parzate (nabam) plus Zn sulfate	3.24	2.98	27	208	115	52	128
Thylate	3.25	3.04	27	209	116	50	129
Phygon	3.35	3.08	30	218	118	51	139
			(YOUNGER LEAVES)				
Check	3.23	2.93	26	222	79	40	121
Parzate (zineb)	3.10	2.92	26	214	88	43	129
Parzate (nabam) plus Zn sulfate	3.13	2.91	30	216	74	39	124
Thylate	3.08	2.91	28	210	92	44	137
Phygon	3.23	2.94	34	215	84	44	134

Table 3. Effect of several fungicides on the zinc content of strawberry leaves.

Materials	Zinc content (ppm) of leaves	
	Older	Younger
Check	16	44
Parzate (zineb)	208	155
Parzate (nabam) plus Zn sulfate	203	121
Thylate	17	41
Phygon	26	48
L.S.D. .01	31	20

Experimental Results: There were no significant differences between fungicide treatments in respect to total calcium, magnesium, iron, or potassium, or in the nitrogen fractions (Table 2). Younger leaves contained less calcium ($P = 0.01$) and magnesium ($P = 0.01$) than older leaves. The differences in protein content, while not significant, are suggestive, and are consistent with analyses of duplicate samples of older leaves (two replications) from check, zineb, and nabam-zinc sulfate plots taken on April 3 (not shown in Table 2). These samples contained, respectively, means of 3.18, 3.08, and 3.06 percent protein nitrogen.

There were significant differences in respect to zinc accumulation in the leaf tissues (Table 3). The content of younger leaves from untreated plants and from those treated with dichlone and thiram averaged about 45 ppm; that of older leaves, about 20 ppm. Zinc accumulation in plants sprayed with zineb or nabam-zinc sulfate was three-fold in magnitude in younger leaves and ten-fold in older leaves. Zinc content of younger leaves of plants sprayed with zineb was significantly greater than that of plants sprayed with nabam-zinc sulfate.

DISCUSSION

This work tends to add weight to the hypothesis that repeated applications of the bisdithiocarbamate fungicides may result in increased susceptibility of certain crops to Botrytis gray mold. In addition to tabular data presented here on fruit infection, field observations indicated that older leaves, particularly petioles, of plants sprayed with these materials exhibited a higher incidence of gray mold than did plants not sprayed or did those sprayed with other fungicides.

It has become well established that nabam-zinc sulfate is quite injurious to vegetable crops in south Florida; whereas, zineb only rarely causes injury. In this work, nabam-zinc sulfate caused a marked leaf-chlorosis on strawberries, while that caused by zineb was very slight. Yet the zinc content in the plants sprayed with zineb was actually higher, significantly so in younger leaves, as determined by chemical analyses, than in those treated with nabam-zinc sulfate. This suggests that "nabam injury" must result from something other than zinc toxicity per se, that is, a by-product of the tank-mix reaction. It is becoming well known that the "inert" diluents in commercial preparations actually play a role in respect to disease control and phytotoxicity. The finding of higher levels of zinc in younger versus older leaves of plants that received no zinc in spray form agrees with results of other workers (8) who found zinc to be concentrated in meristematic tissues. This is not surprising since zinc is apparently quite mobile in plant tissues and some evidence has been presented (7) that it plays an important role in hormone (auxin) synthesis. Also, the present work confirms a previous report (3) that zinc occurred in a greater concentration in plants sprayed with zineb than in those sprayed with nabam-zinc sulfate.

The significance of the way in which zinc accumulates in strawberry leaves sprayed repeatedly with the zinc carbamates and the magnitude of this accumulation constitute two interesting questions.

Regarding the first, since the spray materials were directed toward the plant, a logical assumption might be that zinc accumulated as a result of direct absorption through the leaves. The possibility cannot be ruled out, however, of absorption by roots and translocation to the leaves, since much of the spray material was unavoidably deposited on the soil in the vicinity of the root zone. Nevertheless, Emge and Linn (3), working with tomatoes in solution cultures, apparently were able to eliminate absorption by roots as a factor in zinc accumulation resulting from foliar applications of zinc carbamates.

Regarding the second question, the increases in zinc constituted the only appreciable change in leaf composition that could be demonstrated by the analyses employed. It is tempting, then, to associate the increased zinc content with the apparent increased susceptibility to infection by Botrytis gray mold. It is well known that excess substrate levels of trace metals can induce imbalances in key processes that have far reaching influences (4). Certainly one would expect some aberrations, even though slight, to occur as a result of ten-fold accumulations of a metabolically active metal nutrient. And, if an alteration in metabolism does result, is it not conceivable that susceptibility to a disease organism, in this case gray mold, so intimately connected with and dependent on its host, might be altered also?

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THE WEST VIRGINIA SPOT-ROT COMPLEX OF APPLE IN 1956[1]

R. E. Adams and S. E. Tamburo[2]

Abstract

Results are given for 15,478 isolation attempts from apple fruits with lesions differing from easily identified bitter rot, rust, scab, sooty blotch, and fly speck lesions. Twenty-four different genera of fungi and several kinds of bacteria were isolated. Two fungi, Sordaria fimicola and Trichoderma viride, and the bacteria proved non-pathogenic. The remaining fungi have been reported by others as apple-fruit pathogens or were found to infect apple fruits in this study. The symptoms produced on inoculation into sound fruits are described. Species which produced spots or rots on apple fruits were found in four genera not reported previously. They are, Nigrospora sphaerica, Chaetomium spp., Melanconium fuligineum, and Hypoxylon mediterraneum. The symptom areas from which the fungi were isolated are described, together with the nature of the lesions induced following inoculation of mature fruits. In many cases different fungi could be found associated with similar lesions, so that diagnosis of the causative agent based solely upon the appearance of the symptoms was difficult or impossible.

INTRODUCTION

This study was originally designed to determine the incidence, severity, distribution and symptomatology of Botryosphaeria (Dothiorella) rot (3) and black rot (5) of apple in West Virginia, because the authors experienced difficulty in identifying these diseases solely on the basis of symptoms. In the course of these studies other organisms were found to be present in fruits exhibiting symptoms that have been attributed to Sphaeropsis and Botryosphaeria. It became evident that a thorough investigation was needed to determine the relative importance of fruit rotting pathogens in current methods of apple culture. The purpose of this paper is to report on the frequency of occurrence and pathogenicity to apple fruits of organisms isolated from lesions of unknown etiology.

MATERIALS AND METHODS

Diseased fruits were collected from September until the end of harvest from the major apple growing regions in West Virginia. The fruits were picked from trees just before harvest and from packing lines immediately after harvest. Fruits with bitter rot, rust, scab, sooty blotch, and fly speck were not collected because these diseases are easily recognized. All other fruits exhibiting spot or rot symptoms were collected. Special emphasis was placed on the following varieties which are of commercial importance in West Virginia: York Imperial, Rome Beauty, Red Delicious, Golden Delicious, Grimes Golden, and Stayman.

Blocks of diseased apple tissues, approximately 4 cubic millimeters, were cut and immersed in 95 percent ethyl alcohol for 5 seconds, flamed, and immediately placed in Petri plates on an agar medium containing malt extract 10 grams, yeast extract 2 grams, agar 20 grams and distilled water 1 liter. The pH was adjusted to approximately 6.0 before autoclaving. These plates were incubated in constant temperature rooms at 20°, 25°, and 28°C. Examinations of the cultures were made every 2 days to permit the transfer of slow growing organisms. Records were made of the type of rot and apple variety from which isolations were made.

Cultures representing the different isolates obtained were used to inoculate apple fruits; reisolations were made to complete the rules of proof of pathogenicity. Before inoculation, sound fruits of the varieties Stayman, Grimes Golden, North Western Greening, Golden Delicious, York Imperial, Rome Beauty, Kendall, Newfane, Yellow Newtown, and Jonathan were rinsed in tap water. The fruits were then placed in 0.00125 gauge plastic bags or in moist chambers. Those in plastic bags were surface-sterilized by filling the bag with sufficient 1:000

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Table 1. Organisms isolated from apple spots and rots in West Virginia, and their frequency of isolation in 1956.

Organism	Numbers of times isolated	Organism	Numbers of times isolated
Alternaria spp.[c]	3120	Melanconium fuligineum[a]	120
Nigrospora sphaerica[a]	2885	Cytospora sp.[c]	105
Sphaeropsis malorum	2245	Rhizopus nigricans[c]	85
Botryosphaeria ribis	669	Sporotrichum malorum[c]	80
Cladosporium (2 spp.)[c]	645	Hypoxylon mediterraneum[a]	65
Coniothyrium fuckelii[c]	615	Botrytis cinerea[c]	55
Cylindrosporium sp.	445	Aspergillus (5 spp.)[c]	44
Chaetomium (3 spp.)[a]	435	Cephalothecium roseum[c]	30
Epicoccum granulatum[c]	420	Penicillium expansum[c]	20
Helminthosporium papulosum	340	Trichoderma viride[b]	40
Fusarium (3 spp.)[c]	295	Sordaria fimicola[b]	140
Phoma pomi	275	Bacteria[b]	40
Monilia (2 spp.)[c]	215	Isolation attempts that yielded no organism	1840
Helminthosporium sp.	210		

[a] Not previously reported as apple fruit pathogens.
[b] Proved non-pathogenic.
[c] Commonly reported as storage rots.

mercuric chloride to cover the fruit. The solution was removed after approximately 25 seconds and the fruit and inside of bag rinsed with three changes of sterile distilled water. Fruits to be placed in sterile moist chambers were processed in a similar manner. Inoculations were made by placing spores or mycelium of the organism to be tested on wounded areas of the fruit. Wounds were made by stabbing the fruit with a sterile Number 00 insect pin. Inoculated fruits were incubated in the sealed plastic bags or in moist chambers at room temperature. Observations of the inoculated fruits were made at 2- to 5-day intervals.

EXPERIMENTAL RESULTS

In the course of this study, 15,478 isolation attempts were made. The fungi isolated and the frequency of their isolation are presented in Table 1. Species of Alternaria were isolated most frequently. Nigrospora sphaerica and Sphaeropsis malorum were found almost as frequently. Over 60 percent of all isolations yielded these fungi. Eleven percent of the isolation attempts were sterile (no growth). The remaining isolations yielded Botryosphaeria ribis and species of 20 other genera.

Two fungi, Sordaria fimicola and Trichoderma viride, and all the bacteria proved non-pathogenic when inoculated into healthy fruits. The remaining fungi proved pathogenic to mature apple fruits. Species in four genera were isolated that have not been reported elsewhere as apple fruit pathogens. They were Nigrospora sphaerica, Chaetomium globosum, C. funicolum, C. trilaterale, Melanconium fuligineum, and Hypoxylon mediterraneum. All other genera have been reported to contain species which are apple fruit pathogens (1, 2, 3, 4, 5, 6, 7).

The relative rarity with which Botryosphaeria ribis was found in fruits that exhibited the characteristic symptoms described for this disease (1) is striking. Also, it is of interest that five species of Aspergillus found in the field produced rots that were similar to that caused by Penicillium expansum. The symptoms and the fungi which were obtained from varieties exhibiting these symptoms are presented in Table 2. It is evident that the identification of the causal agent of an apple fruit rot or spot on the basis of symptoms is most difficult. The most reliable means of identification is to induce sporulation of the fungus on the diseased tissue or in pure culture. With further experience it may be possible to develop a diagnostic key for each variety of apple that will permit a grouping of causal agents by symptoms.

Table 2. Fungi isolated and the symptoms on fruits of the different apple varieties from which they were obtained.

Fungus	Apple variety				
	York Imperial	Rome Beauty	Red Delicious	Golden Delicious	Stayman
Alternaria spp.	SNHR[a]	SNH	SNR	HR	NHR
Cylindrosporium sp.	SH	SH			
Epicoccum granulatum	S	SNH		H	SR
Nigrospora sphaerica	SNH	SNH	SNH	SH	SNHR
Fusarium spp.	SH			HR	
Cladosporium elatum	SNHR	N	S		SN
C. cladosporioides	SNHR	N	S		SN
Phoma pomi	SH	N			H
Coniothyrium fuckelii	H	H		R	
Botryosphaeria ribis	SH	HR		H	NR
Sphaeropsis malorum	SNHR	SNHR	H	HR	NR
Cytospora sp.	H				
Chaetomium globosum	S	N		NH	
C. funicolum	S	N		NH	
C. trilaterale	S	N		NH	
Helminthosporium papulosum	H				
Penicillium expansum	H		N	H	
Cephalothecium roseum		R			
Monilinia fructicola		H	R	N	R
Monilia sp.		H	R	N	R
Hypoxylon mediterraneum		R		R	
Sporotrichum malorum		S			
Aspergillus wentii		N			
A. clavatus		S			
A. niger					S
A. elegans				R	
A. fumigatus			N		
Botrytis cinerea					R
Rhizopus nigricans					R
Melanconium fuligineum				H	
Helminthosporium sp.				R	

[a] S = Discolored area that was red, green or darkened, but neither necrotic nor haloed. N = Spot on fruit showing obvious necrosis with no halo. H = Necrotic spot with red, green or brown halo. R = Rot.

Symptoms Shown by Sound Mature Fruits Following Inoculations at Needle Puncture

All inoculated fruits had been held in refrigerated storage for 1 to 3 months before inoculation. The varieties inoculated with each organism are presented in Table 3. The authors realize that symptoms, and perhaps pathogenicity, may differ when young developing apple fruits are inoculated.

Alternaria tenuis Nees -- Black spots developed within 2 days on the varieties Grimes Golden, North Western Greening, and Golden Delicious. These spots were uneven in outline and sunken. Haloes were occasionally produced around the necrotic areas. These spots developed into well defined rots within a period of 4 days after spot symptoms were observed. The rotted areas were approximately circular in outline and were extremely dark (blue-black). Dark, circular spots occurred on Rome Beauty. Within 4 weeks these spots progressed to soft, wet rots. On the varieties Stayman, Jonathan, Kendall, Newfane, and York, rots devel-

Table 3. Symptoms[a] expressed by apples when inoculated with the fungi isolated.

Fungus Species	Apple variety inoculated									
	York Imperial	Rome Beauty	Stayman	Golden Delicious	Grimes	North Western Greening	Jonathan	Kendall	Newfane	Yellow Newton
Alternaria tenuis	R	NR	R	NHR	NHR	NHR	R	R	R	HR
Alternaria sp.	R	R	R	R	R	R	R	R	R	R
Cylindrosporium sp.	NR	NR	NR	N	NR	NR	NR	-	N	-
Epicoccum granulatum	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Nigrospora sphaerica	NHR	NHR	NHR	NHR	NHR	NHR	NHR	NHR	NHR	-
Fusarium spp.	R	R	R	R	R	R	R	R	R	R
Cladosporium elatum	NR	NR	NR	NR	NR	NR	NR	N	N	-
C. cladosporioides	NR	NR	NR	NR	NR	NR	NR	N	N	-
Phoma pomi	NR	NR	NHR	NR	NHR	NHR	NHR	NR	-	-
Coniothyrium fuckelii	NR	R	NR	R	R	NR	R	R	-	-
Botryosphaeria ribis	R	R	R	R	R	R	R	R	R	R
Sphaeropsis malorum	R	R	R	R	R	R	R	R	R	R
Cytospora sp.	N	N	N	-	N	N	N	N	-	-
Chaetomium 3 spp.	R	R	R	R	R	R	R	R	R	R
Helminthosporium papulosum	N	N	N	N	N	N	-	N	-	-
Penicillium expansum	R	R	R	R	R	R	R	R	R	-
Cephalothecium roseum	R	R	R	R	R	R	R	R	R	R
Monilia 2 sp.	R	R	R	R	-	R	-	-	-	-
Hypoxylon mediterraneum	NR	NR	N	NR	NHR	NR	NR	-	N	-
Sporotrichum malorum	R	R	R	R	R	R	-	-	-	-
Aspergillus 5 spp.	R	R	R	R	R	R	R	R	R	-
Botrytis cinerea	R	R	R	-	-	R	-	-	-	-
Rhizopus nigricans	-	R	R	-	R	R	-	-	-	-
Melanconium fuligineum	NR	NR	NR	NR	NHR	NR	NR	NR	-	NR
Helminthosporium sp.	-	NR	NR	-	NR	NR	-	-	-	-

[a] N= Necrotic spot. H = Necrotic spot with halo. R = Rot. - = Not inoculated.

oped immediately with no spot reaction. Within 6 days, over one-half the area of each fruit rotted, becoming dark brown to black and soft. Conidia were evident on the surface of the diseased area. On York Imperial, occasional green haloes surrounded the rotted area. On Yellow Newtown, red haloes developed around small, dark, necrotic spots. After 7 days, a definite rot developed on both varieties. Persistent red rings were evident on the surface of the diseased area.

Alternaria sp. -- This species falls within the grouping Alternaria brevicatenatae Neerg. (8). Inoculated fruits rotted completely within 6 days. The rot was tan to dark brown, with a sharp line of demarcation between diseased and healthy tissue at the advancing margin of the rot. All varieties produced similar symptoms following inoculation. No spots were produced on the inoculated fruits.

Aspergillus niger v. Tiegh., A clavatus Desm., A. elegans Gasparini, A. wentii Wehmer, A. fumigatus Fres. -- A search of the literature has failed to reveal reports of A. clavatus, A. elegans, A. wentii, or A. fumigatus causing apple fruit rots. The rot produced on all varieties inoculated was light tan and firm, and the skin of the diseased area was papery in texture. Sporulation of all five species occurred on the diseased areas when the apple fruits were held in a humid environment.

Botrytis cinerea Pers. -- The rot incited by this organism on all varieties inoculated

was reddish-tan, soft and watery. Sclerotia were produced on the diseased areas in several instances. In the advanced stages of the disease, the fruits become mummified. The mummies retain a reddish-brown coloration.

Cephalothecium roseum (Fr.) Cda. -- The rot produced on all varieties tested produced a firm tan rot after inoculation. In all cases pink spore masses were produced on the diseased areas.

Chaetomium globosum Kunze, C. funicolum Cke., C. trilaterale Chivers. -- These three species of Chaetomium incited similar rots on the fruits inoculated. The rot was tan to brown and the skin of the diseased area was papery. Surrounding the diseased area was a band or halo of glassy-green to glassy-brown tissue. The rot progressed rapidly, completely rotting the fruits within 4 days. The initial symptom produced in many instances was an alteration of the skin pigment in the inoculated area, which occurred within 1 to 2 days after inoculation. On the red and green varieties the area became yellow; on the yellow varieties the area became green.

Cladosporium elatum, C. cladosporioides (Fr.) De Vries -- Necrotic spots appeared in 1 to 4 days after inoculation. The spots were black and slightly sunken. These spots developed into rots in 4 to 10 days. The rot was dark tan to brown and the skin was papery in texture. Occasionally, gray-green or pale green spores were produced on the lesion.

Coniothyrium fuckelii Sacc. -- Initial symptoms on inoculated fruits were limited black sunken spots. Some of these spots progressed rapidly to rots which were either tan in color with a papery textured skin or a black firm rot. Both symptom complexes were produced with the same inoculum; and sometimes even on opposite sides of the same fruit different symptoms were expressed.

Cylindrosporium sp. -- Inoculations with this fungus produced dark necrotic spots which were usually sunken. Occasionally, brown rots with a papery epidermis developed. However, the rots did not develop for a period of 19 or more days after inoculation. The symptoms produced were similar on all varieties inoculated.

Cytospora sp. -- This organism was lost in transfer before taxonomic determination to species could be made. The only symptoms produced on inoculated fruits were sunken, black, necrotic spots. No rot developed during a period of 27 days, after which the fruits were discarded.

Epicoccum granulatum Penz. -- A tan, soft rot developed within 5 days on all varieties inoculated. The rot was dry, and progressed rapidly. The rotted areas became wrinkled. In all cases, the first symptom produced was a spot which rapidly developed into a rot. Clearing of skin pigment was observed following several inoculations into Grimes Golden. The yellow pigment was lost, leaving a rather small zone of green skin.

Fusarium (3 species) -- All three species produced a soft watery rot on all varieties 4 to 7 days after inoculation.

Helminthosporium papulosum Berg. -- Berg (2) gives an excellent description of the symptoms produced by this organism. The spots produced were shiny black, raised or sunken, and limited.

Helminthosporium sp. -- This fungus produced limited necrotic spots on the varieties inoculated. These spots progressed rather slowly, eventually developing a tan rot with a papery textured skin over the rot.

Sporotrichum malorum Kidd & Beaum. -- The initial symptoms were small, shallow, roughly circular light brown areas. The rot progressed rapidly; after 5 days adjacent spots coalesced to form large rotted areas.

Nigrospora sphaerica Mason -- Necrotic spots, necrotic spots with red or green haloes, and distinct rapid rots were produced on all varieties of apple inoculated. Spots and rot were produced on different areas of a single fruit inoculated at the same time with the same isolate. The rot was soft, tan to black in color and had a papery skin.

Phoma pomi Pass. -- Slightly sunken necrotic spots developed within 6 days after inoculation. The spots were usually slow to develop into rots. However, occasionally a rot developed in 4 days.

Melanconium fuligineum (Scribn. & Viala) Cav. -- Necrotic spots were produced on all varieties inoculated. The spot was black with a shiny appearance and slightly sunken. These spots developed into rots within 4 to 8 days. The rot was black and firm. The rot did not involve over one-quarter of the surface of the fruit.

Hypoxylon mediterraneum (de N.) J. H. Miller -- Sunken, necrotic, tan spots which were irregular in outline developed on all varieties inoculated. Occasionally, a green halo developed around the spots on the variety Grimes Golden. The rot produced was brown, soft and watery,

with conidial fructifications on the surface of the diseased tissue. This fungus was compared with the conidial stage of a known culture of H. mediterraneum and agreed with it in all major characteristics.

Fruit symptoms following inoculation with Botryosphaeria ribis, Sphaeropsis malorum, Monilinia fructicola, Penicillium expansum, and Rhizopus nigricans have been described elsewhere and are not described here (1). It was not possible to differentiate between Botryosphaeria rot and black rot on the basis of symptoms.

DISCUSSION

Upon examination of the apple fruits discarded from packing lines, it was estimated that 90 percent or more were discarded because of diseases incited by the organisms reported above or because of bruising.

The great number of different fungi isolated is not too surprising as the mature apple fruit is a good culture medium for many fungi. However, there is the implication that our present spray programs are not adequate for the control of these organisms.

Fewer diseases were found on the variety Red Delicious than on the other varieties. Because of the higher value of Red Delicious, growers spray this variety more thoroughly and pick it promptly when it is mature. The other varieties are neither sprayed as thoroughly nor picked as promptly upon maturity.

More work is necessary to determine the relative importance of these fungi in present day apple culture. The life history of Nigrospora sphaerica is not known. If this fungus is as important as the results of this study indicate, a thorough understanding of its life cycle is essential. It is generally true that the control of a known causative organism can be more specifically directed than the control of a symptom complex of unknown or varying cause.

The results shown in Table 1, which present the organisms isolated from fruit taken directly from the trees, confirm the general supposition that some infection by many storage rot organisms takes place in the field.

To date, it has been our experience that it is not possible to identify the causal organism of a spot or rot on the basis of symptoms. This is especially true concerning Botryosphaeria rot, black rot, and the rot caused by Nigrospora sphaerica.

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BLACKBERRY ANTHRACNOSE CONTROL AND ITS RELATION TO YIELD

F. Ben Struble and Lou S. Morrison

Summary

Over a 5-year period it was demonstrated that blackberry anthracnose (Elsinoë veneta (Burk.) Jenkins) in central Oklahoma could be controlled with fungicides. Liquid lime sulfur and Bordeaux mixture were used, each on different plots, in delayed dormant applications. Bordeaux mixture and ferbam were used separately in later sprays. Most years, a single delayed dormant spray was about as effective in disease control as one or more additional sprays. In none of the 5 years was there an increase in either fruit yield or fruit size as a result of disease control.

Over a period of years there have been several reports dealing with control of anthracnose (Elsinoë veneta (Burk.) Jenkins) of bramble fruits (1, 2, 3, 4, 5, 6). Most of these reports have dealt specifically with anthracnose on raspberries. Liquid lime sulfur, Bordeaux mixture, and lime sulfur followed by ferbam have all been reported as providing satisfactory control of this disease. On black raspberry the principal effect of anthracnose has been to reduce fruit yield and shorten the productive life of the plants. There are few reports dealing specifically with anthracnose on blackberries (5).

During blackberry harvest in 1950 in Pottawatomie County, Oklahoma, where the commercial blackberry plantings were then approximately 800 acres, it appeared that anthracnose was responsible for reducing yields. In 1951 experimental plots were established in this area to determine the efficiency of several fungicides in anthracnose control, the number of fungicide applications necessary for disease control, and the effect of disease control on blackberry fruit yields.

MATERIALS AND METHODS

The area used in these experiments was selected within an established blackberry planting of several acres[1]. The variety was Lawton, the one most commonly grown in Oklahoma. In the original experiment each treatment was replicated three times in a completely randomized design. Each plot consisted of 60 feet of row. Within rows plots were separated by three guard plants; between rows plots were separated by two guard rows, one on each side. Plants were spaced approximately 3 feet apart in the row with 9 feet between rows.

Liquid lime sulfur (8-100) was used in delayed dormant applications on certain plots and Bordeaux mixture (8-8-100) was used in delayed dormant applications on certain other plots. Bordeaux (6-8-100) and Fermate (2 pounds per 100 gallons) were used separately in pre-bloom, post-bloom, and post-harvest treatments. The post-harvest application was used in 1951 only. Certain plots received only a delayed dormant spray, other plots had a delayed dormant plus a pre-bloom spray, and still others had a delayed dormant plus a pre-bloom and a post-bloom or post-harvest spray. Thus some plots received three fungicide applications, others two, and still others only one. The controls received no fungicide. Each plot received the same treatment for each of the 3 years in this experiment. The sequence and number of fungicide applications are indicated in Table 1.

To determine the effectiveness of the various treatments in disease control, lesion counts were made on fruiting laterals just prior to harvest. In 1951, 25 random laterals per plot were used for this purpose. In 1952 and 1953 the number of laterals on which lesions were counted was reduced to 20. After harvest, lesion counts were made on the new canes, that is, those which would produce the next season's crop. For this purpose 20 random canes per plot were used.

To obtain fruit yield data each plot was picked and recorded separately at each of the several pickings necessary throughout the harvest season. Fruit size data were obtained in 1952 and 1953 by weighing 150 random berries at each picking from each plot. Fruit yield and size data presented are averages per treatment for each of the seasons involved.

[1] The authors wish to acknowledge the cooperation of C. F. Tootle, Route 4, Shawnee, Oklahoma, who provided the area necessary for this work.

Table 1. Summary of blackberry anthracnose control trials for the period 1951 through 1953.

Treatment	Average lesions per fruiting lateral			Average lesions per new cane			Average yield per treatment			Average weight 150 berries	
	1951	1952	1953	1951	1952	1953	1951	1952	1953	1952	1953
	No.	No.	No.	No.	No.	No.	Qts.	Qts.	Qts.	g.	g.
Lime sulfur Bordeaux Bordeaux	.9	.2	13.9	2.6	1.0	1.9	14.6	14.4	13.9	241	264
Lime sulfur Fermate Fermate	.6	.4	15.3	1.6	.7	3.5	20.8	20.0	20.1	248	259
Bordeaux Fermate Fermate	.6	1.0	11.9	1.7	1.4	6.6	18.4	23.4	20.2	257	281
Bordeaux Bordeaux Bordeaux	.7	.4	5.0	1.4	.7	2.9	19.2	18.5	21.1	257	257
Lime sulfur Fermate	.9	.6	14.8	3.6	1.5	4.4	20.1	18.1	20.8	239	250
Lime sulfur Bordeaux	.6	.7	11.7	1.7	6.5	2.0	17.8	17.0	16.5	235	262
Bordeaux Fermate	.7	.7	17.3	1.6	1.1	8.1	22.4	22.8	20.9	270	249
Bordeaux Bordeaux	.7	.7	13.2	3.4	4.8	6.3	19.3	19.3	22.2	271	247
Bordeaux	1.6	1.3	13.8	3.5	6.2	12.4	22.8	20.7	19.8	278	274
Lime sulfur	1.1	.6	22.5	4.0	4.8	13.9	18.2	19.8	14.1	239	236
None	5.6	9.3	28.6	7.1	23.2	73.8	20.0	18.3	16.8	266	259
L.S.D. 5%	1.26	1.05	N.S.	1.83	4.53	13.45	N.S.	N.S.	N.S.	N.S.	N.S.
L.S.D. 1%	1.72	1.43	N.S.	2.50	6.17	18.35	N.S.	N.S.	N.S.	N.S.	N.S.

Table 2. Blackberry spray trials for anthracnose control, 1954-1955.

Treatment	Average lesions per fruiting lateral		Average lesions per new cane		Average yield per treatment		Average weight 150 berries	
	1954	1955	1954	1955	1954	1955	1954	1955
	No.	No.	No.	No.	g.	g.	g.	g.
Bordeaux, delayed dormant only	3.8	1.9	59.7	84.9	3555	2159	275	372
Bordeaux, 4 applications	1.1	1.3	5.4	19.7	3297	1969	254	375
None	6.8	2.4	106.7	89.8	3859	2662	269	372
L.S.D. 5%	1.34	.55	23.26	33.05	N.S.	N.S.	N.S.	N.S.
L.S.D. 1%	1.84	.75	31.86	45.27	N.S.	N.S.	N.S.	N.S.

After 3 years, when it was apparent that the disease could be controlled but that control was not being reflected in fruit yield, it was decided that further trials should emphasize the effect of disease control on yield. Subsequently the whole experiment was revised. The area in the field in use was consolidated in an attempt to eliminate as many variables as possible. The number of treatments was reduced to three. These were Bordeaux mixture applied at the delayed dormant, pre-bloom, post-bloom, and post-harvest stages; Bordeaux applied at delayed dormant only; and no fungicide.

Each of these three treatments was replicated 10 times in a randomized block design. In each plot there were 10 plants with 3 guard plants between plots in the row. A single guard row was left between plot rows. Data on numbers of lesions, fruit yields, and fruit size were taken from each of 10 tagged canes, one per plant, per plot.

RESULTS AND CONCLUSIONS

Results from the first 3 years of this work are presented in Table 1; those from the last 2 years are in Table 2. It will be noted from these data that disease as measured by lesion counts was significantly reduced on both fruiting laterals and new canes by any of the fungicide treatments. One exception to this occurred in 1953 when poor control resulted on the fruiting laterals. At least for the first 3 years, control resulting from a single delayed dormant fungicide application was about as good as that obtained when two or three additional applications were made. In the 1954-55 experiments better control, especially on new canes, resulted from a total of four fungicide applications than from a single delayed dormant application.

The data in Tables 1 and 2 further show that in no year was disease control reflected in an increase in fruit yield or fruit size. It is possible that during the period of these experiments the disease level with which we were dealing was not sufficiently high to influence yield. There is also the possibility that certain unfavorable environmental factors may have been obscuring the effects of disease on yield. For at least three of the years involved, 1952 through 1954, temperatures were excessively high during the period when fruits were ripening and being harvested. High temperatures in addition to dry weather at or near harvest tend to restrict fruit size and thus reduce yields. In both 1952 and 1953 rainfall immediately preceding and during harvest was very light. Rainfall distribution and totals, however, for the years 1950 through 1955 were not appreciably different from each other during those periods when infection might

be expected to occur.

From the data presented it can be concluded that under the conditions of these experiments anthracnose was controlled but fruit yield or size was not increased as a consequence of control.

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NEW HOST RECORDS FOR MELOIDOGYNE HAPLA INCLUDING TWO PLANTS NATIVE TO CALIFORNIA

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Summary

The host range of Meloidogyne hapla is extended by the addition of Bush Germander, Teucrium fruticans L.; California Laurel, Umbellularia californica Nutt.; and Salt Rush, Juncus leseurii Boland. The last two plants are native to California and their locations suggest that M. hapla may also be native to this State.

Three new host records have been added to the list of plants attacked by the root-knot nematode, Meloidogyne hapla. Bush Germander, Teucrium fruticans L., grown as an ornamental in Davis, California was found heavily infected with this species of root-knot nematode.

The California Laurel, Umbellularia californica Nutt., and Salt Rush, Juncus leseurii Boland., also were found infected by M. hapla in localities that have never been under cultivation. Both of these plants are native to California soils which would suggest that M. hapla may also be native to this State.

California Laurel was found infected with root-knot nematodes in two locations. The first, found on February 24, 1949, was on the bank of an intermittent stream located near Vacaville, Solano County. The second sample was collected near Jenner, Sonoma County, August 31, 1951 on the bank of the Russian River.

The infection on Salt Rush was collected May 21, 1955 near Dillon Beach, Marin County. These plants were growing near the edge of a shelf ending approximately 30 feet above the surf. There was no evidence that this land had ever been cultivated. It was currently being used for grazing sheep.

The identifications of these nematode collections were made from perineal sections as well as host range tests. Perineal sections were made from root-knot females found in the host plant roots at all three locations. Also, a nematode population was built up in the greenhouse over a period of approximately 10 months by growing tomatoes in the soil from Dillon Beach. This was used as inoculum for host range tests which were repeated to confirm the results. The test plants were grown in sterilized soil to which an aliquot of the inoculum was added for 56 and 91 days respectively for the two series. After that time the roots were washed clean and examined for galls. Perineal sections of females from all the positive hosts were made and compared to the original sections made from field collections. These were all identified as M. hapla, which was further corroborated by the host tests.

Positive infections of the roots were found on:

Alfalfa	--	California common	Pepper	--	World Beater
Carrot	--	Danvers Half Long		--	Hungarian Yellow Wax
Clover	--	Ladino	Lycopersicon peruvianum		
Geranium			Strawberry	--	Shasta
Onion	--	Southport White Globe	Tomato	--	Pearson Improved
Peanut	--	Spanish		--	Rutgers
			Tobacco		

Negative results were obtained from:

Barley	--	Atlas S-53	Garlic		
Corn	--	Kingcrost KR-2	Watermelon	--	Klondike
Cotton			Wheat		

These results all conform to the host range of M. hapla as reported by Sasser (2).

Knowledge concerning the original source of infestations of our common plant parasitic nematodes is important to direct control programs dealing with established populations of

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these pests as well as to preventive measures of a regulatory nature designed to restrict movement and further distribution of the parasites. In areas of long established agriculture it is usually difficult or impossible to trace infestations of nematodes to their native habitats. The collection of nematode species on native plants in areas with no history of cultivation could be considered as evidence for the natural distribution of such species in that region.

Chitwood and Birchfield (1) recently reported evidence of this nature from which they conclude Tylenchulus semipenetrans is apparently native to Florida soils. There is a possibility that the root-knot infections on California Laurel described above were carried to those host plants from distant agricultural areas by stream water originally washing through infested fields. However, it is highly unlikely that the infection of Salt Rush at Dillon Beach would have occurred in this way and this collection is reported as evidence that M. hapla may be native to California soils.

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SOME GREENHOUSE TESTS WITH THE NEMATICIDES PRD AND ORD[1]

Shelby L. Brothers and J. R. Christie[2]

Abstract

When mixed with nematode-infested soil at the rate used, the nematicidal efficacy of PRD compared favorably with that of Nemagon, was somewhat better than that of ORD, and was considerably better than that of V-C 13. Both chemicals were phytotoxic, PRD somewhat more so than ORD. Malathion had little or no effect on the nematodes present. The presence of the chemicals in the soil did not prevent Hoplolaimus coronatus Cobb and certain other species from penetrating the roots of corn. H. coronatus was the most difficult of the plant nematodes to kill, while Belonolaimus sp. and Trichodorus christiei Allen were comparatively easier to kill with the chemicals tested. Miscellaneous soil inhabiting nematodes as a group were not easier to kill than were the plant parasitic species.

Two prospective nematicides, designated PRD and ORD, have been made available for testing. PRD is identified chemically as 3, 4-dichlorotetrahydrothiophene 1, 1-dioxide. ORD is an isomer of PRD.

According to information furnished by the manufacturer[3], PRD is a white crystalline solid essentially odorless and heat stable (boiling point 186° C). The chemical is 0.22 percent soluble in water at 20° C. Experiments have shown that PRD acts as a contact nematicide against nematodes inhabiting the soil. It does not have systemic properties and will not kill nematodes within living roots. It does not kill root-knot nematode eggs nor has it proven effective as a fungicide.

The purpose of this report is to record the results of several greenhouse experiments, the objects of which were to obtain additional information (a) on the efficacy of these chemicals in killing different kinds of plant nematodes, and (b) on their phytotoxicity.

Nematicidal Efficacy

Briefly stated, these experiments consisted of mixing the chemicals at specified rates into nematode-infested soil and, after stated intervals of time, examining samples of the treated soil to determine the extent to which the nematodes had been killed. Certain other materials were included for comparison.

Sandy soil of the Leon series was obtained from a field at the Central Florida Experiment Station at Sanford. It harbored a fairly high population of miscellaneous nematodes of which Hoplolaimus coronatus Cobb was the predominating plant parasitic species with lesser numbers of Belonolaimus sp. and Trichodorus christiei Allen. Enough soil for each experiment was placed on a concrete floor and mixed to bring about as even a distribution of the nematode population as possible. The chemicals were mixed with the soil mechanically, and the experiments were conducted in six-inch clay pots.

Rates of application, expressed in amounts per cubic foot of soil, were: PRD and ORD, 20 grams; V-C 13, 5 milliliters; malathion, 3 milliliters; Nemagon, 1 milliliter. These rates apply to the formulations as received, not to active ingredients. PRD and ORD were powders each containing 10 percent active ingredient; V-C 13, malathion, and Nemagon were emulsifiable concentrates containing 75 percent, 50 percent, and 50 percent active ingredients, respectively.

The suggestion has been made that even though a chemical may not actually kill nematodes, it may perhaps inactivate them to such an extent that they are unable to penetrate roots. To test this hypothesis, corn was planted in each of the pots at the beginning of the experiment im-

[1] Adapted from a thesis by the senior author presented to the Graduate Council of the University of Florida in partial fulfillment of the requirements for the degree of Master of Science.

[2] Graduate student, College of Agriculture and Nematologist, Agricultural Experiment Station, respectively, University of Florida.

[3] Diamond Alkali Company.

Table 1. Effect of V-C 13, Nemagon, DRD, ORD, and malathion on the nematode population of the soil and the number of nematodes penetrating the roots of corn.

Living Nematodes Recovered	Days after Applications			
	28	35	42	49
Untreated Controls (Soil)				
Belonolaimus sp.	21	29	33	13
Criconemoides spp.	26	19	19	8
Hoplolaimus coronatus	318	259	489	336
Trichodorus christiei	55	74	66	5
Others	322	231	205	98
Total	742	612	812	460
Untreated Controls (Roots)				
Hoplolaimus coronatus	399	163	...	...
Others	170	217	...	...
Total	569	380	...	...
V-C 13, 5 ml./cu. ft. (Soil)				
Belonolaimus sp.	10	1	1	82
Criconemoides spp.	47	32	14	70
Hoplolaimus coronatus	359	131	94	175
Trichodorus christiei	1	9	0	0
Others	147	105	176	275
Total	564	278	185	602
Percentage Reduction (all spp.)	24	54	77	...
V-C 13, 5 ml./cu. ft. (Roots)				
Hoplolaimus coronatus	571	108	...	...
Others	374	218	...	...
Total	945	326	...	...
Nemagon, 1 ml./cu. ft. (Soil)				
Belonolaimus sp.	0	0	1	0
Criconemoides spp.	0	1	35	1
Hoplolaimus coronatus	17	6	2	6
Trichodorus christiei	0	0	22	0
Others	188	120	142	100
Total	205	127	202	107
Percentage Reduction (all spp.)	73	80	76	77
Nemagon, 1 ml./cu. ft. (Roots)				
Hoplolaimus coronatus	12	1	...	...
Others	157	101	...	...
Total	139	102	...	...

Table 1. (Continued).

Living Nematodes Recovered	Days after Applications 28	35	42	49
PRD, 20 g./cu. ft. (Soil)				
Belonolaimus sp.	0	1	0	1
Criconemoides spp.	2	7	2	12
Hoplolaimus coronatus	37	37	23	140
Trichodorus christiei	0	0	1	0
Others	58	178	90	166
Total	97	212	116	319
Percentage Reduction (all spp.)	88	66	86	30
PRD, 20 g./cu. ft. (Roots)				
Hoplolaimus coronatus	49	106	...	...
Others	56	97	...	...
Total	105	203	...	...
ORD, 20 g./cu. ft. (Soil)				
Belonolaimus sp.	3	0	1	0
Criconemoides spp.	0	8	9	8
Hoplolaimus coronatus	46	58	80	130
Trichodorus christiei	0	0	1	0
Others	74	173	132	113
Total	123	239	223	251
Percentage Reduction (all spp.)	84	62	73	46
ORD, 20 g./cu. ft. (Roots)				
Hoplolaimus coronatus	65	53	...	...
Others	87	231	...	...
Total	152	284	...	...
Malathion, 3 ml./cu. ft. (Soil)				
Belonolaimus sp.	31	6	0	47
Criconemoides spp.	41	12	8	8
Hoplolaimus coronatus	557	185	320	567
Trichodorus christiei	42	10	0	9
Others	527	138	197	255
Total	1198	351	525	886
Percentage Reduction (all spp.)	0	44	36	0
Malathion, 3 ml./cu. ft. (Roots)				
Hoplolaimus coronatus	478	342	...	...
Others	261	141	...	...
Total	739	483	...	...

mediately after the chemicals were applied.

After stated intervals of time, the soil was removed from the pots. The soil from each pot was mixed separately to bring about a more uniform distribution of the nematodes, and a 150-milliliter sample taken. The living nematodes were removed from this sample by the sieving-Baermann technic and counted. The roots of the corn plants from each pot were washed vigorously to remove all nematodes that might be adhering to the surface, then macerated in a blender. The living nematodes were removed from the resulting pulp by the sieving-Baermann technic and counted. Results are given in Tables 1 and 2. The experiments recorded in Table 1 were replicated, i. e., each count is the mean of two 150-milliliter samples, one from each of two pots. The experiments recorded in Table 2 were not replicated and each count is that of one sample.

Table 2. Effect of PRD on the nematode population of soil and on the number of nematodes penetrating the roots of corn.

Living Nematodes Recovered	Days after Applications 31	38	45	52
Untreated Controls (Soil)				
Belonolaimus sp.	36	55	62	93
Criconemoides spp.	26	37	6	10
Hoplolaimus coronatus	81	178	75	151
Trichodorus christiei	17	75	32	68
Others	163	304	125	328
Total	323	649	300	650
Untreated Controls (Roots)				
Hoplolaimus coronatus	144	205	187	101
Others	169	233	166	398
Total	313	438	353	499
PRD, 20 g./cu. ft. (Soil)				
Belonolaimus sp.	0	0	2	0
Criconemoides spp.	3	8	7	0
Hoplolaimus coronatus	11	5	9	4
Trichodorus christiei	1	0	0	0
Others	67	37	19	89
Total	82	50	37	93
Percent Reduction (all spp.)	75	92	87	86
PRD, 20 g./cu. ft. (Roots)				
Hoplolaimus coronatus	31	7	2	12
Others	1719	975	266	117
Total	1750	982	268	129
Untreated Controls (Soil)				
Belonolaimus sp.	30	23	17	7
Criconemoides spp.	7	16	1	5
Hoplolaimus coronatus	349	448	82	196
Trichodorus christiei	49	101	245	421
Others	271	298	204	1081
Total	706	886	549	1710
Untreated Controls (Roots)				
Hoplolaimus coronatus	37	100	16	12
Others	610	1108	176	114
Total	647	1208	172	126
V-C 13, 5 ml./cu. ft. (Soil)				
Belonolaimus sp.	5	5	0	1
Criconemoides spp.	4	8	0	4
Hoplolaimus coronatus	120	195	70	36
Trichodorus christiei	2	5	1	2
Others	44	80	159	133
Total	175	293	230	176
Percentage Reduction (all spp.)	75	66	58	83
V-C 13, 5 ml./cu. ft. (Roots)				
Hoplolaimus coronatus	31	7	2	12
Others	1719	975	266	117
Total	1750	982	268	129

Table 3. Effects of PRD and of ORD on the average number of days required for the highest percentage of germination of squash, bean, and okra seeds when planted the day of application, and 7 and 14 days after application.

Rates grams per cu.ft.	Days Between Application and Planting					
	0		7		14	
	Percent Germination	Days Required	Percent Germination	Days Required	Percent Germination	Days Required
PRD on Squash						
Untreated Control	95	12	85	10	85	15
20	85	16	90	15	95	18
40	95	19	85	14	75	18
80	70	23	90	22	90	18
PRD on Bean						
Untreated Control	100	9	100	8	100	11
20	90	21	55	12	95	16
40	45	25	100	15	100	18
80	0		50	19	60	19
PRD on Okra						
Untreated Control	80	16	70	22	85	19
20	85	19	65	21	75	20
40	40	21	80	22	75	19
80	20	18	75	24	65	22
ORD on Squash						
Untreated Control	60	13	96	12	60	10
20	73	16	96	12	93	10
40	76	14	96	12	93	12
80	83	16	100	13	96	12
ORD on Bean						
Untreated Control	100	13	96	12	90	12
20	100	13	90	12	90	11
40	93	13	93	12	100	11
80	96	13	86	13	73	12

In no instance does there appear to be any consistent difference between counts made 28 days (Table 1) or 31 days (Table 2) after the chemicals were applied and those made at later dates.

Combining all data for each chemical, regardless of time intervals or kinds of nematodes, PRD reduced the general nematode population of the soil by a mean of 78 percent, Nemagon by a mean of 76 percent, ORD by a mean of 68 percent, and V-C 13 by a mean of 61 percent.

Combining all data for different kinds of nematodes, regardless of chemical or time intervals, the populations of Belonolaimus sp. were reduced by a mean of 95 percent, those of Trichodorus christiei by a mean of 94 percent, those of Hoplolaimus coronatus by a mean of 75 percent, and those remaining miscellaneous nematodes by a mean of 54 percent. The above percentage reduction for Belonolaimus sp. does not include one high count of 82 that probably was an experimental error (V-C 13, 49 days after application)

Phytotoxicity Tests

These experiments were conducted in much the same manner as those to determine nematicidal efficacy. The soil was of the same type, but it harbored very few plant parasitic nema-

FIGURE 1. Effects of PRD (above) and of ORD (below) on germination and plant growth when seeds were planted the day of treatment. Horizontal rows from top to bottom (above), beans, squash, and okra, (below) beans and squash. Vertical rows, left to right, (1) untreated control, (2) 20 grams per cubic foot, (3) 40 grams per cubic foot, (4) 80 grams per cubic foot. Photographed 21 days after seeds were planted.

todes. PRD and ORD were the only materials included, and they were tested at three rates: 20, 40, and 80 grams per cubic foot of soil. This is equivalent to 2, 4, and 8 grams, respectively, of active ingredient. The experiment was conducted in 10-inch clay saucers and included two replicates. The experiment with PRD included three test plants: corn, bean, and okra. In the experiment with ORD, okra was omitted. Ten seeds were planted in each saucer, and plantings were made immediately after, 7 days after, and 14 days after the chemical was applied.

Results are shown in Table 3 wherein percentage of germination and days required to germinate are, in each case, based on the behavior of 20 seeds.

The effects of the chemicals on the growth of the test plants, when seeds were planted the same day the chemicals were applied, is shown in Figure 1. An interval of 7 days between applications and planting reduced stunting to some extent and an interval of 14 days reduced it still more. After an interval of 14 days, the 20 gram rate of ORD stunted the plants only very slightly.

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VAPAM INEFFECTIVE AGAINST FUSARIUM WILT OF TOMATO

A. G. Plakidas[1]

It has been demonstrated by several investigators that Vapam (sodium N-methyldithiocarbamate) possesses fungicidal properties in addition to its being an effective nematocide and herbicide. It has been tested extensively in this laboratory as a soil drench in seedbeds of various vegetables, and it has given excellent control of damping-off fungi in addition to killing nematodes and weed seeds. However, information is still lacking on the activity of Vapam against specific soil-inhabiting fungus pathogens. This paper presents evidence which indicates that Vapam is ineffective against the tomato wilt fungus, Fusarium oxysporum f. lycopersici (Sacc.) Snyder & Hansen, even when used at a higher strength than that usually recommended.

The test plot used is a part of the writer's garden, and comprises an area of 605 square feet surrounded by a metal barrier (galvanized iron sheet sunk to a depth of 10 inches to keep Bermudagrass and nutgrass out). It is high and well-drained; no run-off water gets into it. The soil was heavily infested with root-knot nematodes and the wilt fungus (Fusarium oxysporum f. lycopersici). In the 1956 season, carrots, okra, cucumbers, and tomatoes grown on this plot were severely affected with root knot. Of 44 Marglobe tomatoes nine were affected with wilt. The wilted plants were scattered throughout the planting, not limited to a particular spot.

In late September, 1956, the area was prepared carefully as a seedbed. Vapam, diluted at the rate of 1 quart to approximately 3 gallons of water in a sprinkling can, was applied evenly to a measured area at a rate of 1 quart per 64 square feet. The area was then sprinkled with a garden hose until the soil was wet to a depth of about 6 to 8 inches. One month after treatment, two rows of lettuce and one row of carrots were planted in one end of the plot, and the rest left fallow until the last week of March, 1957, at which time 35 tomato plants of the Marglobe variety and 12 of a wilt-resistant selection (unnamed) were planted in four short adjoining rows. Six hills of cucumber and eight pepper seedlings were also planted at the same time, and a row each of okra and pole beans a month later. The tomato and pepper transplants had been grown on steam-sterilized soil in flats in the greenhouse. The stakes used for tying the tomato plants were sterilized by methyl bromide gas because they had been used on wilt and nematode infested soil the previous season and it was feared that soil adhering to them might carry the wilt fungus or nematodes or both.

RESULTS AND DISCUSSION

Seemingly complete control of nematodes resulted from the treatment. To date (July 15) no trace of root knot has been found on any of the nematode-susceptible crops (carrot, lettuce, bean, cucumber, pepper, tomato, okra) grown on the treated soil. Excellent control of weeds, both broadleaves and grasses, also was obtained. The surface of the soil remained practically bare during fall and winter.

No control of fusarial wilt of tomato was obtained. Of 35 Marglobe plants 19 wilted and died by the end of June. Isolations were made from the affected plants and Fusarium was obtained in culture in every case. None of the 12 plants of the wilt-resistant variety wilted. A comparison of the incidence of wilt in the 1956 season (untreated soil) with that of the 1957 season (Vapam-treated soil) on the same plot indicates that the treatment actually promoted an increase in the population of the wilt fungus. In 1956, 9 out of 44 plants (20.4 percent) were infected; in 1957, 19 out of 35 plants (54.3 percent) were infected. It is not believed that the treated soil became reinfested with the wilt fungus from some outside source. The metal barrier separating the treated soil from the surrounding area and the precautions taken not to introduce any contaminated material make reinfestation from outside sources unlikely. It is concluded that Vapam did not eradicate the wilt fungus from the soil, although it probably reduced the population initially, and that, in the absence of competition from other organisms less resistant to the fumigant, the wilt Fusarium population eventually increased to even a

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higher level than that existing in the soil before fumigation. Wilson[2], investigating the effect of several chemicals applied to the soil for possible control of radish yellows (caused by Fusarium oxysporum f. raphani Kendr. & Snyder), states that "there is often a rather rapid 'build-up' toward the pre-treatment population levels of the causal organism of the disease and that the rate at which this occurs varies with different soil fungicides". Incidentally, in Wilson's experiments, Vapam at the rate of 50 gallons per acre did not eliminate the radish yellows Fusarium from the soil, and this is taken as an indication that other wilt Fusaria, in addition to that of tomato, are tolerant of Vapam.

DEPARTMENT OF BOTANY, BACTERIOLOGY AND PLANT PATHOLOGY, LOUISIANA STATE UNIVERSITY, BATON ROUGE, LOUISIANA

[2] Wilson, J. D. 1956. Seek method to control radish disease caused by soil fungus. Ohio Farm and Home Research 41: 52-54.

CONTROL OF DOWNY MILDEW OF BROCCOLI WITH ANTIBIOTICS AND FUNGICIDES[1]

John J. Natti

Summary

Germination of spores of Peronospora parasitica was completely inhibited at concentrations of 12 ppm or less by Actidione, ayfactin, candicidin, endomycin, filipin, fungichromin, neomycin sulfate, pleocidin, thiolutin, tricothecin, and tyrothricin; at above 12 to 50 ppm by anisomycin, polycycline and streptomycin sulfate; and at 100 ppm by mycostatin. Amphotericin A, amphotericin B, griseofulvin, oxytetracycline (Terramycin), and tniactin had no effect on spore germination.

In greenhouse tests on control of downy mildew of broccoli by spray applications, anisomycin, mycostatin, streptomycin sulfate and thiolutin were the most effective of the antibiotics tested. Endomycin, filipin, fungichromin, and neomycin also provided control of downy mildew. Manzate and Spergon SL were the most effective of the fungicides tested in the greenhouse.

Control of downy mildew with streptomycin was of both a surface protective and a topical systemic nature. Streptomycin sprays caused bleaching of the broccoli foliage.

In field tests, Agri-Strep at 3.0 pounds per acre provided outstanding control of downy mildew of broccoli as long as 27 days after the final spray application. This treatment caused severe bleaching of the foliage. Agri-Strep at 0.6 pounds per acre was more effective than Manzate at 4 pounds per acre and Spergon SL at 3 pounds per acre but caused moderate bleaching of the foliage. Copper-Zinc and Copper Manganese were the most effective of the fungicides tested, but caused a trace of injury. On the basis of control and freedom from phytotoxicity, Manzate and Spergon SL were the most promising of the fungicides. Thioneb also showed some promise. Captan 50W, Kemate and Vancide M were ineffective.

INTRODUCTION

Downy mildew caused by Peronospora parasitica Pers. ex Fr. is one of the major diseases of broccoli in New York. All commercial broccoli varieties tested by Natti et al. (1) were susceptible to downy mildew, although differences in susceptibility within and between varieties were observed. The only method of control at the present time appears to be the application of protective sprays or dusts to the broccoli foliage. The application of a fungicide with the insecticide spray has not provided effective control (1, 2). One of the main reasons for lack of effective control appeared to be the inadequate coverage of the foliage with the fungicide during periods favorable for disease development. Because spray residues are readily removed from the glabrous surface of broccoli foliage during rains, and also because spray applications are generally postponed until a few days after the rains have ceased, the times at which protection is most necessary are the times at which the broccoli foliage is least protected. A fungicide that would provide protection against downy mildew by being absorbed and distributed systemically within the plant would eliminate the problem of lack of protection during periods favorable for disease development. In a search for such a systemic protectant, a number of antibiotics were tested for control of downy mildew of broccoli. This report contains results of laboratory tests on the influence of antibiotics on germination of the spores, and of greenhouse and field spray tests on control of downy mildew.

MATERIALS AND METHODS

The antibiotics and fungicides tested are listed in Table 1.

[1] Approved by the Director for publication as Journal Paper No. 1084. New York State Agricultural Experiment Station, Geneva, New York.

Table 1. Antibiotics and fungicides used in tests on control of downy mildew of broccoli.

Antibiotics	Active Ingredient	Source
Actidione	cycloheximide	The Upjohn Company
Actidione derivatives:	cycloheximide acetate	"
	" oxime	"
	" semicarbazone	"
Agrimycin 100	15% streptomycin plus 1.5% oxytetracycline	Chas. Pfizer and Co., Inc.
Agri-Strep	37% streptomycin sulfate	Merck and Company
Amphotericin A		E. R. Squibb and Sons
Amphotericin B		"
Anisomycin		Chas. Pfizer and Co., Inc.
Ayfactin		Bristol Laboratories
Bacitracin		Commercial Solvents Corp.
Candicidin		Merck and Company
Endomycin		The Upjohn Company
Etamycin		Bristol Laboratories
Filipin		The Upjohn Company
Fungichromin		Merck and Company
Griseofulvin		"
Mycostatin		E. R. Squibb and Sons
Neomycin sulfate		S. B. Penick and Co.
Phytomycin	20% streptomycin nitrate	Olin Mathieson Chemical Corp.
Pleocidin		Merck and Company
Polycycline		Bristol Laboratories
Streptomycin sulfate		Merck and Company
Terramycin	oxytetracycline	Chas. Pfizer and Co., Inc.
Thiactin		Bristol Laboratories
Thiolutin		Chas. Pfizer and Co., Inc.
Tricothecin		Imperial Chem. Industries, Ltd.
Tyrothricin		S. B. Penick and Co.
Fungicides		
Captan 50W	50% captan	Stauffer Chemical Co.
Captan 90W	90% captan	"
Copper-Zinc	48% Cu and 4% Zn	Tennessee Corporation
Copper-Manganese	48% Cu and 3.5% Mn	"
Kemate	50% dyrene	Chemagro Corporation
Manzate	maneb	E.I. duPont de Nemours & Co., Inc.
Spergon SL	chloranil	Naugatuck Chemical
Thioneb	a polyethylene thiuram sulfide	"
Vancide M (Niacide A)	manganous dimethyldithiocarbamate, benzothiazyl disulfide, thiram, and manganous benzothiazylmercaptide	Niagara Chemical Division

Spore Germination Tests

The effectiveness of a number of antibiotics in inhibiting germination of spores of the downy mildew fungus was determined in the laboratory. A dilution series of each antibiotic was prepared and 15 ml of each concentration were added to a Petri dish containing 5 ml of a spore suspension of Peronospora parasitica (50,000 spores per ml). The diluting effect of the spore suspension on the concentration of the antibiotics was adjusted in the preparation of the antibiotic dilutions. The Petri dishes were covered and placed in an incubator maintained at 60° F. After 6 hours of incubation, the number of germinated spores from a total of 100 was determined. Duplicate tests were conducted.

Table 2. Influence of antibiotics on germination of spores of Peronospora parasitica in in vitro tests.

Antibiotic	Concentration of active ingredient in ppm					
	32	16	8	4	2	1
	Percentage of spores germinated[a]					
Actidione (cycloheximide)	0	0	0	0	0	0
Candicidin	0	0	0	0	0	0
Pleocidin	0	0	0	0	0	0
Tricothecin	0	0	0	0	0	12
Endomycin	0	0	0	0	2	7
Fungichromin	0	0	0	0	19	38
Neomycin sulfate	0	0	0	0	28	39
Ayfactin	0	0	0	0	29	41
Filipin	0	0	0	11	21	59
Thiolutin	0	0	0	10	26	48
Streptomycin sulfate	0	0	52	49	73	79
Etamycin	39	62	63	79	84	81
Mycostatin	26	69	81	68	78	84
Streptomycin nitrate	29	38	49	59	86	79
Polycycline	38	36	66	79	84	82
Thiactin	57	74	79	85	88	89
Griseofulvin	76	77	84	79	89	86
Terramycin (oxytetracycline)	81	69	87	88	74	85
Spergon SL	0	0	0	0	7	23
Check (water)	88% germination					

[a]Percentage based on total of 200 spores observed from each treatment.

Table 3. Influence of antibiotics on germination of spores of Peronospora parasitica in in vitro tests.

Antibiotic	Concentration of active ingredient in ppm						
	100	50	25	12	6	3	1.5
	Percentage of spores germinated[a]						
Endomycin	0	0	0	0	0	0	2
Neomycin sulfate	0	0	0	0	0	2	18
Thiolutin (pure)	0	0	0	0	0	32	61
Ayfactin	0	0	0	0	4	31	42
Fungichromin	0	0	0	0	6	56	64
Thiolutin (crude)	0	0	0	0	20	22	60
Tyrothricin	0	0	0	0	49	72	66
Filipin	0	0	0	11	16	81	80
Anisomycin	0	0	0	12	39	48	79
Streptomycin sulfate	0	0	6	17	47	61	71
Polycycline	0	0	57	66	77	72	84
Mycostatin (crude)	0	26	69	67	87	81	77
Amphotericin A	53	66	74	73	82	76	81
Amphotericin B	72	69	83	76	88	82	78
Griseofulvin	56	64	71	84	72	78	69
Thiactin	58	74	69	65	68	78	82
Spergon SL	0	0	0	0	0	3	36
Check (water)	80% germination						

[a]Percentage based on total of 200 spores observed from each treatment.

Greenhouse Tests

Experiments were designed to obtain information on the effectiveness of the treatments as surface protectants, on the retentiveness and stability of the test material on broccoli foliage, and on possible systemic activity. Plants of the broccoli variety Waltham 29 were grown in 6-inch pots, one plant per pot. The plants were first sprayed when they had 6 to 8 leaves, the smallest leaf being at least 2 inches in width. Four plants were sprayed at a time on a turntable rotating at 1 RPM. The sprays were applied for 2 minutes at 25 PSI by means of a DeVilbiss point spray gun. An emulsifiable formulation of parathion at the rate of 1 pint per 100 gallons was added to each spray preparation for the purpose of removing the bloom from the broccoli foliage. Removal of the bloom permitted the ready wetting of the foliage, a condition essential for obtaining uniform coverage of the foliage with the test material and, later, with the inoculum. The plants were sprayed 3 times at 3-day intervals in most instances. To obtain information on the surface protective value of the test material, some of the plants were inoculated 3 hours after the final spray. To obtain information on the retentiveness and stability of the test material, and on possible systemic activity, other plants were not inoculated until 144 hours, or longer, after the final spray. Beginning with the fourth day after the final spray application, the foliage of these plants was washed 3 times daily in the course of the regular greenhouse watering routine.

Spores of the fungus were obtained from broccoli plants maintained in a moist chamber in the greenhouse. A fresh supply of spores was obtained by inoculating broccoli plants every 6 to 7 days. The spores were removed from the broccoli foliage by immersing the leaves in distilled water and brushing the spores free from the mycelium. The suspension of spores was then filtered through two thicknesses of cheesecloth to remove mycelium, germinated spores and plant debris. The concentration of the spore suspension was determined by means of a haemocytometer and adjusted to contain 50,000 spores per ml.

The broccoli plants were inoculated by atomizing 4 plants at a time for 2 minutes with the spore suspension by means of a paint spray gun adjusted to deliver the inoculum at 10 PSI. Immediately after inoculation, the plants were placed in the saturated atmosphere of a moist chamber in the greenhouse where they were held for 4 to 6 days. The plants were then removed from the moist chamber to a greenhouse bench where they were scored for downy mildew infection 5 days later.

The effectiveness of the treatments in controlling downy mildew was determined by rating the severity of infection of the test plants according to the following scale: 1 = no infection; 2 = 1 to 9 lesions per square inch of leaf area; 3 = 10 to 24 lesions; 4 = 25 to 49 lesions; 5 = 50 to 99 lesions; and 6 = 100+ lesions, leaf wilted or abscised. Data were taken from 5 leaves of each plant starting with the third expanded leaf from the tip of the plant.

Field Test

One field test on control of downy mildew by spray applications was conducted in 1956. Plants of the Waltham 29 broccoli variety were started in a seedbed and transplanted to the field on July 20. Each treatment plot consisted of two adjacent 25-plant rows. Each treatment was replicated 4 times. The plots were separated by a single buffer row. The sprays were applied at the desired rate in 100 gallons of water per acre at 40 PSI by means of a hand-operated sprayer equipped with a cylinder of compressed CO_2. The plots were sprayed on August 31; September 4, 12, and 19. Because of the low incidence of downy mildew, the sprays were discontinued on September 19 to permit the development of the disease. Another spray application of each fungicide was made on October 8 for the purpose of obtaining samples of heads for spray residue analyses. No streptomycin sprays were applied after the application of September 19. The number of lesions in an area 3 inches square in the center of each of 25 leaves per plot was counted on September 20, October 3 and 18. The leaves to be scored were selected at random from the central portion of the plant.

RESULTS

Laboratory Tests

Actidione, candicidin, and pleocidin at 1 ppm; tricothecin at 2 ppm; ayfactin, endomycin, fungichromin, and neomycin sulfate at 4 ppm; filipin and thiolutin at 8 ppm; and streptomycin

Table 4. Control of downy mildew of broccoli 3 hours and 144 hours after final spray application. Greenhouse, 1956.

Treatment [a]	Hours between final spray and inoculation	
	3	144
	Mildew Index [b]	
Ayfactin	4.1	4.4
Bacitracin	5.3	4.6
Endomycin	3.8	4.6
Etamycin	4.6	5.3
Fungichromin	3.1	4.0
Mycostatin	3.1	2.6
Neomycin sulfate	4.5	4.4
Streptomycin sulfate	2.6	2.6
Thiolutin	2.3	2.2
Spergon SL	1.0	2.2
No fungicide	4.4	4.6
LSD at 5% level	0.4	0.6
1% level	0.5	0.8

[a]Plants sprayed 3 times at 4-day intervals ; sprays contained active ingredient at 100 ppm.
[b]Mildew Index is the average index of 5 leaves per plant from 4 plants per treatment. Mildew Index indicates severity of infection according to the following scale: 1 = no infection; 2 = 1 to 9 lesions per square inch of leaf area; 3 = 10 to 24 lesions; 4 = 25 to 49 lesions; 5 = 50 to 99 lesions; and 6 = 100^{+} lesions, leaf wilted, or leaf abscised.

Table 5. Control of downy mildew of broccoli 3 hours and 144 hours after final spray application. Greenhouse, 1956.

Treatment [a]	Conc. (ppm)	Hours between final spray and inoculation	
		3	144
		Mildew Index [b]	
Anisomycin	500	1.0	1.0
	250	1.0	1.0
Endomycin	500	1.3	2.6
	250	2.4	4.4
Filipin	500	1.7	2.4
	250	2.0	4.0
Mycostatin	500	1.0	2.3
	250	1.3	2.5
Neomycin sulfate	500	2.5	3.0
	250	3.1	3.7
Streptomycin sulfate	500	1.0	1.7
	250	2.0	2.9
Thiolutin (crude)	500	2.0	3.0
	250	2.4	3.7
Spergon SL	500	1.0	1.3
	250	1.0	2.3
No fungicide	---	5.3	5.5
LSD at 5% level		0.5	0.9
1% level		0.7	1.3

[a]Plants sprayed 3 times at 3-day intervals; 2 plants per treatment.
[b]Mildew Index - see Table 4.

sulfate at 16 ppm inhibited the germination of all spores of the mildew fungus (Table 2). At a concentration of 32 ppm, etamycin, mycostatin, streptomycin nitrate, polycycline, and thiactin inhibited the germination of 50 to 60 percent of the spores while griseofulvin and Terramycin had no effect on the germination of the spores. Spergon SL, which was used as the standard for comparison between tests, completely inhibited germination of the spores at 4 ppm, but a low percentage of spores germinated at 2 ppm.

In a second test, endomycin at 3 ppm; neomycin sulfate and thiolutin (pure) at 6 ppm; ayfactin, fungichromin, thiolutin (crude), and tyrothricin at 12 ppm; filipin and anisomycin at 25 ppm; streptomycin sulfate and polycycline at 50 ppm; and mycostatin (crude) at 100 ppm inhibited the germination of all spores (Table 3). Amphotericin A, amphotericin B, griseofulvin and thiactin were ineffective at 100 ppm. Spergon SL inhibited germination of all spores at 6 ppm and of a high percentage of the spores at 3 ppm. Some of the antibiotics also inhibited the germination of a high percentage of the spores at concentrations lower than that at which complete inhibition occurred. Anisomycin at all concentrations at which germination occurred caused the malformation of the germ tubes.

Greenhouse Tests

Endomycin, fungichromin, mycostatin, streptomycin sulfate and thiolutin at 100 ppm reduced the severity of downy mildew of broccoli plants which had been inoculated 3 hours after the third and final spray; whereas ayfactin, bacitracin and neomycin sulfate provided no control (Table 4). On broccoli plants inoculated 144 hours after the final spray, only mycostatin, streptomycin sulfate and thiolutin gave a significant reduction in the severity of downy mildew. Spergon SL, the fungicide used as the standard in the greenhouse spray tests, gave complete control on plants inoculated 3 hours after the final spray. On plants inoculated 144 hours after the final spray mycostatin, streptomycin and thiolutin were equally as effective as Spergon SL.

Anisomycin at 250 and 500 ppm provided complete control on plants inoculated 3 hours and on plants inoculated 144 hours after the final spray (Table 5). This antibiotic was moderately injurious at both dosages. At 250 and 500 ppm, endomycin, filipin, mycostatin, neomycin sulfate, streptomycin sulfate and thiolutin reduced the severity of downy mildew on plants inoculated 3 hours and on plants inoculated 144 hours after the final spray (Table 5). Mycostatin and streptomycin sulfate were the most effective of these antibiotics. Control of downy mildew provided by these materials was comparable to that provided by Spergon SL. Mycostatin caused a slight flecking of the foliage and streptomycin sulfate caused bleaching of the foliage at both concentrations.

In greenhouse tests, Actidione at 1, 5 and 10 ppm was ineffective in controlling downy mildew and was phytotoxic even at 1 ppm. The Actidione derivatives, cycloheximide oxime and cycloheximide semicarbazone, at 5, 10 and 20 ppm were ineffective, but the derivative cycloheximide acetate provided some surface protective action. None of the derivatives exhibited systemic activity; the derivatives were non-phytotoxic at 20 ppm.

The addition of glycerol at the rate of 1 percent by volume of the spray enhanced the effectiveness of streptomycin in controlling downy mildew. Maintaining plants in a saturated atmosphere after they were sprayed also increased the effectiveness of streptomycin. The bleaching of the foliage of broccoli plants was more severe from treatments with streptomycin plus glycerol than from treatments with streptomycin alone, and also from treatments in which the plants were maintained in a saturated atmosphere after they were sprayed. The combination of glycerol together with maintaining the plants in a saturated atmosphere after spray applications resulted in a very severe bleaching of the foliage. Downy mildew lesions were not observed in the bleached portions of the leaves even though lesions were present in green areas of the same leaves.

Some of the fungicides reported to be effective against downy mildew diseases of various crops were tested in the greenhouse for control of downy mildew of broccoli. Captan 90W, Manzate and Spergon SL were the most effective of the fungicides on plants inoculated 3 hours after the final spray application (Table 6). Streptomycin sulfate at 200 ppm was moderately effective but was ineffective at the lower concentrations. On plants inoculated 144 hours after the final spray, Manzate and Spergon SL were more effective than Captan 90W, particularly at the lower concentrations (Table 6). Spergon 2-100 was equally as effective as Manzate and more effective than Captan 90W at 2-100. At 1/2-100 and 1-100, Manzate was the most effective of the fungicides. Streptomycin sulfate alone was ineffective but streptomycin sulfate plus glycerol was as effective as Manzate. Copper-Zinc and Copper-Manganese, although only

Table 6. Control of downy mildew of broccoli with fungicides and with streptomycin 3 hours and 144 hours after final spray. Greenhouse, 1956.

Treatments [a]	Lb./100 gal.	Hours from final spray to inoculation: 3	144
		Mildew Index [b]	
Spergon SL	2.0	1.6	1.7
	1.0	1.9	3.9
	0.5	3.7	3.8
Captan 90W	2.0	2.0	2.4
	1.0	3.1	5.2
	0.5	3.4	5.1
Manzate	2.0	1.7	2.1
	1.0	2.3	2.5
	0.5	2.5	2.7
Copper-Zinc	2.0	3.7	2.3
	1.0	3.7	3.3
	0.5	3.8	3.3
Copper-Manganese	1.0	3.5	3.5
	0.5	4.2	3.7
Streptomycin sulfate	200 ppm	3.0	4.8
	100 "	3.7	5.2
	50 "	4.2	5.3
Streptomycin sulfate + glycerol [c]	200 "	3.0	2.6
	100 "	3.6	2.6
	50 "	4.0	4.1
No fungicide		4.5	5.4
LSD at 5% level		0.5	0.6
1% level		0.7	0.8

[a] Plants sprayed 3 times at 3-day intervals, 4 plants per treatment.
[b] Mildew Index -- see Table 4.
[c] Glycerol added at rate of 1 percent by volume of spray.

moderately effective, appeared to have considerable residual activity.

The combination of Copper-Zinc plus Agrimycin did not exhibit any synergistic action in controlling downy mildew of broccoli.

Streptomycin sulfate in several tests provided effective control of downy mildew as long as 288 hours after the final spray, at which time the tests were concluded. Control was of a systemic nature but appeared for the most part to be localized within the sprayed leaves. Some streptomycin was translocated to the first few leaves above the uppermost sprayed leaf but the protection provided by streptomycin was primarily of a topical systemic nature. Thiolutin did not exhibit systemic activity in similar tests.

Field Tests

Downy mildew was not observed in the spray plots until the first week of September. On September 20, one day after the fourth spray application, downy mildew was present throughout the entire planting but was not severe in any of the plots. The incidence of mildew on September 20 was significantly less in the sprayed plots than in the unsprayed (Table 7). Because of the low incidence of mildew, no material was outstanding in providing control. On October 3, 14 days after the last spray application, differences in severity of downy mildew in the treatment plots were apparent. Plots sprayed with Agri-Strep at 3.0 pounds per acre (a concentration of approximately 1000 ppm of streptomycin) were almost completely free from downy mildew regardless of whether the Agri-Strep was applied alone or with glycerol (Table 7). Agri-Strep at 0.6 pounds per acre, Copper-Zinc, and Copper-Manganese ranked next in order of effectiveness, followed by Manzate, Spergon SL and Thioneb. Captan 50W, Vancide M and Kemate were not effective under the conditions of this test. The plots were scored again

Table 7. Control of downy mildew of broccoli in field spray tests.

Treatments [a]	Lbs./Acre [b]	Number of Downy Mildew Lesions per leaf [c]	
		Sept. 20	Oct. 3
Agri-Strep	3.0	0.3	3.0
Agri-Strep plus Glycerol [d]	3.0	0.4	2.6
Agri-Strep	0.6	3.3	25.2
Agri-Strep plus Glycerol	0.6	2.6	17.5
Copper-Zinc	6.0	1.1	22.5
Copper-Manganese	6.0	1.7	27.2
Spergon SL	3.0	1.6	36.8
Manzate	4.0	2.0	37.5
Thioneb	6.0	2.0	43.5
Captan 50W	6.0	3.3	47.4
Vancide M	4.0	2.2	47.9
Kemate 50%	6.0	2.1	50.7
No spray	---	11.1	54.7
LSD at 5% level		1.1	9.3
1% level		1.4	12.5

[a] Plots sprayed on 8/31, 9/4, 9/12 and 9/19.
[b] Sprays applied at rate of 100 gallons per acre.
[c] Average number of lesions in 9 square inches of leaf area of 100 leaves per treatment.
[d] Glycerol added to spray at the rate of 1% by volume of spray.

on October 18 and the results at this date were essentially the same as those on October 3. The significant feature of the scoring made on the latter date was the effective control still being provided by streptomycin 27 days after the final spray.

Agri-Strep at 3.0 pounds per acre caused considerable bleaching of the foliage, particularly when glycerol was added to the spray. The florets of the central stalk of the head of some plants which had been sprayed with streptomycin plus glycerol were pink to purple instead of the normal green. Agri-Strep at 0.6 pounds per acre caused bleaching of the margins of the leaves but had no effect on the florets. Copper-Zinc and Copper-Manganese caused a very fine pitting of the underside of some of the lower leaves. The other fungicides were not phytotoxic. On the basis of freedom from injury and downy mildew control, Manzate and Spergon SL were the most promising materials.

No streptomycin was detected on the surface of broccoli leaves harvested 30 days after the final spray application. The results of assay tests with extracts from leaves sprayed with streptomycin were inconclusive because extracts from unsprayed leaves also exhibited activity against Bacillus subtilis, the test organism. Whatever the mode of action of streptomycin, the results of this field test demonstrated that protection provided by streptomycin against downy mildew was of a systemic nature.

DISCUSSION

The performance of some antibiotics in spore germination tests conducted in the laboratory did not correspond with their performance in controlling downy mildew when applied as sprays in greenhouse tests. For example, Actidione and ayfactin were very effective in inhibiting the germination of spores in the laboratory tests but were ineffective in controlling downy mildew when applied as sprays to broccoli foliage. Other antibiotics such as anisomycin, mycostatin and streptomycin were only moderately effective in inhibiting spore germination but were the most effective materials in controlling downy mildew when applied as sprays. Spore germination tests, therefore, are of little value in selecting antibiotics for control of downy mildew.

Control of downy mildew with streptomycin was of both a surface protective and a systemic

nature. Evidence for surface protection is the control of downy mildew provided by streptomycin on plants inoculated a short interval after spraying. Evidence for action of a systemic nature is the control provided by streptomycin as long as 27 days after the spray applications were discontinued and in the absence of any spray residues on the foliage. This systemic action was restricted for the most part to the leaves actually sprayed. The action of streptomycin can be considered as a localized or a topical systemic type. Control of downy mildew provided by streptomycin can be attributed to direct toxicity of the antibiotic to the fungus or to changes in the metabolism of the broccoli plants induced by streptomycin to render the plants resistant. The absence of downy mildew lesions in bleached areas of the foliage of broccoli plants sprayed with streptomycin may be indicative of changes in metabolism which prevent the synthesis of substrates essential to the downy mildew fungus.

Streptomycin appears to enter the plant by diffusion. Any condition that maintains streptomycin in a soluble form on the foliage favors the entrance of the antibiotic into the plant. Transpiration does not appear to be directly involved in the movement of streptomycin into the plant. On the basis of severity of bleaching of the foliage, more streptomycin entered the plant in a saturated atmosphere in which the rate of transpiration usually is negligible than under conditions of low relative humidity which usually favor high rates of transpiration. Maximum benefits from streptomycin probably can be obtained by spraying foliage when it is wet.

Some translocation of streptomycin occurred to leaves immediately above the uppermost sprayed leaves. However, it does not appear that streptomycin is translocated in sufficient concentration to provide protection of the entire plant.

The bleaching of foliage sprayed with streptomycin appears due to the the gradual disappearance of chlorophyll from the leaves rather than to the sudden destruction of chlorophyll. Streptomycin appears to prevent the conversion of protochlorophyll to chlorophyll so that the supply of chlorophyll is gradually depleted. Corn seeds soaked in streptomycin solutions frequently produce seedlings completely devoid of chlorophyll although protochlorophyll is present in the leaves. These seedlings fail to develop chlorophyll even when exposed to sunlight (unpublished data).

On the basis of disease control and freedom from phytotoxicity, Manzate and Spergon SL were the most promising of the fungicides tested. More data are needed on concentration and timing of spray applications with these materials for effective control. Copper-Zinc and Copper-Manganese warrant further testing. These materials were more effective than Manzate or Spergon SL but were slightly phytotoxic. Residues of Copper-Zinc and Copper-Manganese appeared to be retained on the foliage longer than those of the other fungicides. Captan 50W, Kemate, and Vancide M (Niacide A) provided control during the period at which the sprays were applied but were ineffective 14 days after the final spray application, probably because these materials are readily removed by weathering from broccoli foliage.

The bleaching of broccoli foliage sprayed with streptomycin will discourage the use of this antibiotic for control of downy mildew on broccoli. The outstanding control of downy mildew provided by streptomycin in the field tests, however, indicates the possibilities of antibiotics as systemic protectants for the control of downy mildew. Other antibiotics are being tested for control of downy mildew.

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FUNGICIDE SCREENING TRIAL ON ONION

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Onion is a minor crop in the Everglades. The reason for this is believed to be due, in part at least, to field diseases and storage rots. The most common disease in the field is purple blotch (Alternaria porri). A screening test was set up in the spring of 1957 to study the effect of fungicides on disease development on the Texas Early Grano variety.

Purple blotch failed to develop. Another disease, characterized by a tip dieback, was present in the plots and caused considerable damage before the season was over. Definite causal relations were not established, but a species of Stemphylium was associated with the dieback. Information was obtained on control of dieback and on phytotoxicity.

Table 1. Effect of various fungicides on phytotoxicity and tip dieback and on yields of onions.

Materials	Concentration in 100 gallons (pounds)	Phytotoxicity[a]	Tip dieback[a]	Yield (pounds)
Check	--	--	M	21
Manzate	1 1/2	N	M	24
Dyrene	2	M	M	15
Thylate	1 1/2	N	L	27
Copper A	4	N	M	19
Orthocide 50W	3	L to M	M	16
Fermate	3	N	M	21
Parzate	2	N	L	28
Zerlate	2	N	M	23
Thioneb 50W	2	N	M	22
Phygon	3/4	S	M	7
Nabam plus zinc sulfate	2 quarts plus 3/4 pounds	N	M	22
L. S. D. .05				6
.01				8

[a] N = none, L = light, M = moderate, S = severe.

Twenty-five fungicide spray applications were made on a 5-day schedule from December 5, 1956 through April 1, 1957. Results are shown in Table 1.

Only Parzate and Thylate showed any control of dieback. Phygon caused severe injury, Orthocide light to moderate, and Dyrene moderate injury. Disease control resulted in increased yield, and phytotoxicity reduced the yield. Onion samples were taken from each plot and stored by suspending from rafters in citrus open-mesh shipping bags. Rot was light in all cases. This suggested the importance of aeration during storage.

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ANTHRACNOSE, AN UNDESCRIBED DISEASE OF CELERY IN THE EVERGLADES

R. S. Cox[1]

Abstract

A new disease (anthracnose) of celery is described. The similarity of the causal fungus to other falcate forms, particularly C. truncatum, of the genus Colletotrichum is noted. Tribasic copper sulfate provided excellent control. Other materials used alone were ineffective to moderately good. Parzate and Thylate in combination were highly effective. This suggested synergism.

A disease was noted in October 1956 in the celery seedbed of a commerical planting near Belle Glade, Florida. Symptoms were quite different from those of the common diseases. Isolations, greenhouse pathogenicity studies and response of the disease to pesticides in the field confirmed this difference, and led to the suggestion that the disease is a heretofore unreported anthracnose on celery.

SYMPTOMATOLOGY

Infection appears to be restricted to the leaf blade, suggesting a weak form of parasitism. Symptoms appear initially as small reddish-brown spots. These gradually enlarge in irregular outline to an area comprising 1 to 3 millimeters in diameter (Fig. 1, A). Because of their small size, the spots appear superficial to the naked eye, but examination under magnification reveals that they are sunken (Fig. 1, A). With age the center of the spots becomes whitish in color and paperish in texture (Fig. 1, B). Chlorosis then develops, ultimately encompassing an area several times that of the original spot (Fig. 1, C). Chlorotic areas from adjacent spots coalesce, finally involving the entire leaf blade. Thus, under severe infection, the whole seedbed takes on a yellowish cast, readily discernible from a distance. Seedlings seldom are killed outright although stunting from defoliation may result.

Fruiting bodies of the fungus associated with this disease have not been found on leaves attached to the host plant. They do form on leaves that have reached an advanced stage of decay in the moist chamber. This possibly would suggest further a weak form of parasitism.

ISOLATIONS, MORPHOLOGY, AND GREENHOUSE PATHOGENICITY

Isolations from young lesions yielded rather consistently a Colletotrichum sp. In culture or on infected leaves in the moist chamber, the fungus formed unicellular falcate spores in acervuli. The mean dimensions of 40 conidia were 26.3 x 3.2μ; the range, 20.1-32.5 x 2.8-3.7μ. Comparable measurements for 20 multicellular conidiophores were 21.6 x 2.3μ (mean) and 11.2-35.0 x 1.7-3.0μ (range). The spores, exuded from the acervulus and confined in the mucilaginous matrix characteristic of the genus, though hyaline, exhibit a grayish to pinkish color en masse. Sporulation occurs rather sparsely on potato-dextrose agar, and isolates may entirely lose their ability to sporulate.

Dark brown multicellular setae are produced at random over the acervulus, both in culture and on infected leaves in the moist chamber. Average dimensions of 14 setae were 175 x 6.4μ; the range, 70-345 x 4.8-8.5μ. A further characteristic of the fungus in culture is the production of sterile structures suggestive of immature perithecia.

In greenhouse trials, inoculations were successful only under conditions of extended periods of free moisture and relatively high temperature. Under these conditions typical symptoms were produced, and the fungus was subsequently recovered in culture from the lesions.

[1] Formerly, Associate Plant Pathologist, Everglades Experiment Station. The writer is indebted to Nelson Nelms, field foreman, for supervision of the field work. University of Florida, Agricultural Experiment Station, Journal Series No. 630.

FIGURE 1. Various stages of leaf symptoms of anthracnose on celery: A) early infection; B) and C) advanced stages of infection.

Table 1. Effect of various materials alone and in combination on the control of celery anthracnose and early blight; Belle Glade, Florida, 1956.

Treatment[a]	Concentration		Anthracnose severity[b]		Early blight
	Pounds/ 100 gallons	Ppm	October 12	October 19	
Check	---		3.9	4.5	4.5
Agrimycin-100		100	2.2	3.8	4.0
		50	3.2	4.5	4.0
		25	3.5	4.7	4.5
TBCS	4		1.0	1.0	4.2
Spergon	4		3.2	3.2	0.8
Thylate	1 1/2		2.5	4.0	0.2
TBCS	4				
plus Agrimycin		25	0.0	1.0	2.9
Spergon	4				
plus Agrimycin		25	3.0	3.0	1.0
Thylate	1 1/2				
plus Agrimycin		25	2.9	3.8	0.2
Parzate	2				
plus Agrimycin		25	2.0	2.8	0.0
TBCS	4				
plus Parzate	2				
plus Agrimycin		25	0.2	0.0	0.0
Spergon	4				
plus Parzate	2				
plus Agrimycin		25	2.2	2.0	0.0
Thylate	1 1/2				
plus Parzate	2				
plus Agrimycin		25	0.2	0.2	0.0

[a] Percent active ingredient of materials tested as follows: Agrimycin-100, 15% streptomycin sulfate, 1.5% Terramycin; TBCS (tribasic Copper Sulfate), 53% metallic copper; Spergon, 50% chloranil; Thylate, 65% thiram; Parzate, 65% zineb.
[b] Scored on a 0-11 basis where 11 equals complete defoliation.

IDENTITY OF ORGANISM

A review of the literature failed to reveal a Colletotrichum parasitic on celery, although what is apparently a saprophytic falcate-spore form has been reported on other members of the Umbelliferae (4). Information on identity is too preliminary for the establishment of a positive taxonomic relationship. Nevertheless, a considerable similarity between this fungus and other falcate-spore forms of the genus is evident. For example, it would not be possible to separate the celery fungus from C. truncatum (Schw.) Andrus & W. D. Moore (1) on the basis of available data on morphology. For this reason, instead of attempting an exact taxonomic placement, it is suggested, rather, that comparative evaluation of all the falcate-spore forms of the genus might be rewarding. Only a cursory search is necessary to indicate that a reduction to synonomy of some of the species might be possible[2].

CONTROL

Materials and Methods: Commercial seedbeds (300 feet x 4 feet) planted July 12, 1956 to Kilgore's Florida Green Pascal variety, were divided into plots 10 feet x 4 feet. Four plots were employed per treatment in randomized blocks. Sprays were applied at the rate of 10 gallons to the full-length bed with a hand-operated broom attached to the experimental rig described elsewhere (3). Twenty applications were made between August 31 and October 26.

Results: Results of this test provided further evidence that this disease is different from common troubles of celery (Table 1). In fact, had it not been for the differential response to the various fungicides, the disease probably would have been overlooked. Of the materials tested, tribasic copper sulfate was most effective. This material is relatively less effective against early blight (Cercospora apii). Parzate, which is highly effective against early blight in the seedbed, was only moderately effective against the new disease. This would tend to eliminate the possibility that the new disease was a variant of early blight. Similar evidence differentiating it from bacterial blight (Pseudomonas apii) is provided by the failure of Agrimycin to control the new disease (2).

It is of particular interest to note the effects of Thylate and Parzate on anthracnose. Thylate alone was relatively ineffective (disease index of 4.0) and Parzate only moderately effective (index of 2.8). These two materials in mixture, however, resulted in near-perfect control (index of 0.2)[3]. With such data at hand, the suggestion of synergism is unavoidable.

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[2] A culture of this fungus has been placed in the American Type Culture Collection, 2112 M Street, N. W., Washington, D. C. (Accession No. 12880, unidentified Colletotrichum.)
[3] Agrimycin used in these combinations was not credited with playing a significant role.

CERCOSPORA LEAF SPOT ON CUCUMBERS IN NORTH CAROLINA

N. N. Winstead, L. H. Person, and R. D. Riggs

During the summer of 1957 Cercospora leaf spot caused by Cercospora melonis Cke. was observed on cucumber (Cucumis sativus) in the field in North Carolina. The disease has been observed on cucumber plants in Warren, Franklin, Nash, Wake, Duplin, Wayne, and Halifax Counties. Plants of the fresh market varieties, Ashley, Stono, and Marketer, and the pickling variety Model were affected.

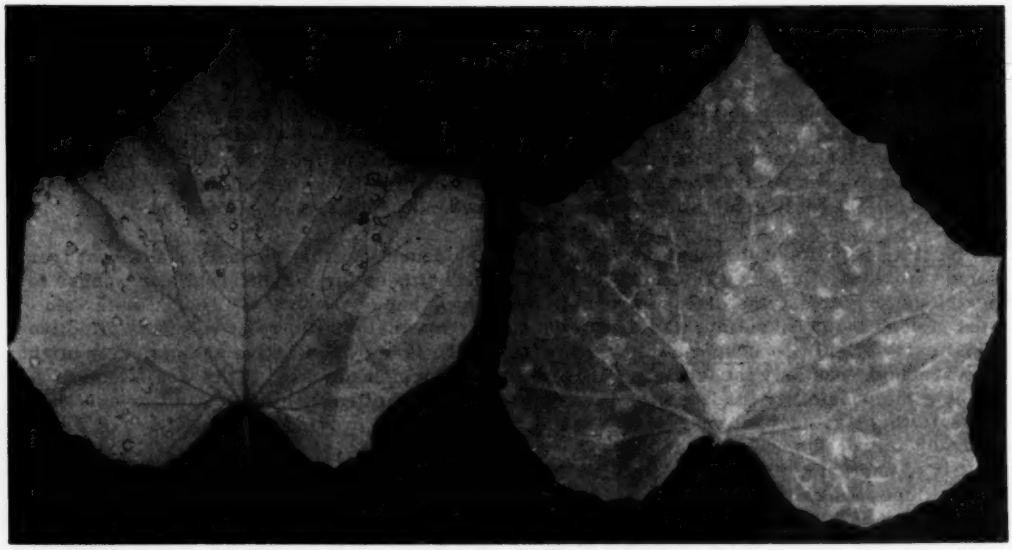

FIGURE 1. Cucumber leaves showing advanced, left, and early, right, symptoms of Cercospora leaf spot.

Lesions at first appeared as small light tan to white spots with a narrow olive-brown margin. These necrotic spots were surrounded by a diffuse chlorotic zone. In the advanced stages the entire leaf became light yellow. Leaves showing early and advanced symptoms are shown in Figure 1.

This is the first report on the occurrence of this disease on cucumber in North Carolina.

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WIND-WHIP ON SNAP BEAN

R. S. Cox

Observations during the past 3 1/2 years have convinced the writer that wind damage results in greater losses to many vegetable crops in the Everglades and surrounding areas year in and year out than any other single factor. A constant reminder of the importance of wind is the breaks (sunflower, field corn) used routinely for such crops as snap bean, pepper, cucumber, etc. The damage incurred is quite diverse in scope, that is, marginal leaf burn (lettuce, snap bean, pepper, tomato, cucumber), and wind scar of fruit (snap bean, tomato). Plantings are also seriously damaged by high winds due to the instability of the plants in the loose muck soils.

FIGURE 1. Wind-whip on the Black Valentine snap bean: left, stem symptoms; right, petiole symptoms. Photography by Henry M. Spelman III.

A situation was observed on snap beans during 1955 that was eventually associated with high winds. Symptoms (Fig. 1) were suggestive of aerial damping-off. Isolations, however, were negative. The damage always showed up in exposed fields following periods of high winds, and field surveys led to the conclusion that the damage was due to a wind-whipping effect on very young succulent seedlings. Stands were reduced seriously, in some instances to such an extent that replanting was necessitated. The name suggested for this physiological disease is Wind-whip.

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A HIGH-TEMPERATURE TOLERANT STRAIN OF HELMINTHOSPORIUM TURCICUM

R. S. Cox and F. E. VanNostran

The seasonal occurrence of northern corn leaf blight (Helminthosporium turcicum Pass.) and southern corn leaf blight (H. maydis Niskado & Miyaka) in south Florida has been reported[1]. Southern blight is primarily a problem in the fall; northern blight, in the winter and spring. Stoner et al.[1] suggested that temperature was the primary contributing factor for this phenomenon. During the period from October 8, 1954 to August 9, 1955 records were kept on the incidence of these two diseases in continuous sweet corn plantings of the Golden Security variety on the Everglades Experiment Station farm. This information, together with evidence pointing to the occurrence of an apparently new high-temperature tolerant strain of H. turcicum, is presented below.

Beginning October 8, H. maydis was by far the dominant species and continued to be dominant through November. By December 12, H. turcicum had built up in incidence almost equal to that of H. maydis. During January, February and March of 1955 southern corn leaf blight was not present in the plots. Northern corn leaf blight was present, but did not build up to any extent. This was attributed to cool, dry weather.

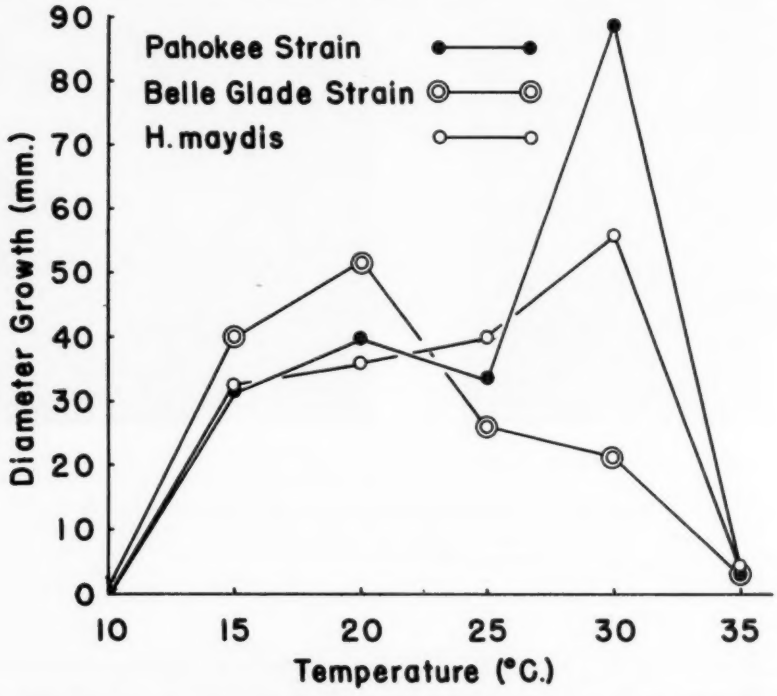

FIGURE 1. Comparison of growth of the Pahokee and Belle Glade strains of Helminthosporium turcicum with growth of H. maydis, at various temperatures.

Southern corn leaf blight appeared in May, but northern blight remained dominant. This relationship continued through June, July and up to August 9. On that date both diseases were present on an older planting; however, on a younger planting immediately alongside, northern blight was completely absent whereas southern blight was present and active. Northern blight

[1] Stoner, Warren N., Robert A. Conover, J. M. Walter, John F. Darby, George Swank, Jr., and F. V. Stevenson. 1954. Control of two Helminthosporium leaf blights of sweet corn in Peninsular Florida. Florida Agric. Exp. Sta. Cir. S-69.

failed to develop subsequently while southern blight became epiphytotic. An examination of the weather records failed to provide a clue for this abrupt change-over. Both the mean weekly minimum and maximum temperatures charted practically straight lines for the entire months of July and August; the minimum temperature fluctuated around 70° F; the maximum, 90° F.

Nevertheless, these observations provided confirmation for the theory that southern blight was basically a fall problem, and northern blight was the primary disease in the spring. Thus, a report, later proved to be accurate, that northern blight was epiphytotic in a single field near Pahokee (10 miles to the north of Belle Glade) during September and October of 1955 came as a considerable surprise. It was again epiphytotic in this same field in September of 1956.

An isolate of the fungus, called the "Pahokee strain", was obtained from the field in 1956. Subsequent comparative temperature studies in vitro of the Pahokee strain and an isolate obtained from the Belle Glade area showed a difference of 10° C for optimal growth (Figure 1). The optimum temperature for the Pahokee strain (30° C) coincided with that of an isolate of H. maydis included in the temperature studies. Another point of interest was the more vigorous growth exhibited by the Pahokee strain (Figure 1).

In summary, the unseasonal occurrence of northern corn leaf blight in the fall seasons of 1955 and 1956, the high temperature tolerance of the associated strain of H. turcicum, and its growth rate in culture indicate that a new strain of the fungus has evolved. If this is true, it could have practical implications in the sweet corn breeding program.

It has been established that one of the primary problems in any breeding program for disease resistance revolves around the variability of the pathogen in question. In this case, the emergence of a new high-temperature tolerant strain may of itself be of no consequence. Nevertheless careful attention should be given to the resistance of breeding lines, as well as to commercial varieties, to this or other new strains of the fungus that may develop.

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A BACTERIAL STALK ROT OF IRRIGATED CORN IN NORTH CAROLINA

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Summary

A bacterial stalk rot has been observed following extensive overhead irrigation of corn at several locations in North Carolina. Leaf and stem tissue is rapidly invaded, becoming tan to brown and water soaked. Parenchymatous tissue is rotted quickly and affected portions of leaves are shredded. Diseased plants frequently topple over at the point of infection. A Gram-negative bacterium with peritrichous flagella was isolated from diseased plants and its pathogenicity to corn was demonstrated in greenhouse inoculations. The causal organism can also cause a pith decay of tobacco and soft rot of potato tubers, carrots, squash, cucumbers, cabbage and onions. Routine biochemical tests indicated that the bacterium can be considered a species of Erwinia, but that it differs from previously described species with respect to one or more biochemical reactions and pathogenicity to corn.

A severe stalk rot of field corn (Zea mays L.) was observed in breeding plots at the McCullers Test Farm of the North Carolina Agricultural Experiment Station in the summer of 1953 and again in the summers of 1954 and 1955. In all instances the appearance of the disease followed a period of extensive overhead irrigation. In 1954 specimens were also collected from a field of irrigated corn near Winston-Salem, North Carolina. The percentage of plants affected by this disease in a given field rarely exceeded 10. However, all diseased plants were either killed or so severely damaged that no ears were formed. There was no evidence of secondary spread from diseased to healthy plants once overhead irrigation was discontinued.

In isolations from material obtained at the McCullers Station and the farm in the vicinity of Winston-Salem, one type of bacterium was consistently isolated from the advanced margin of necrotic areas in the stalk. During the 1956 growing season additional isolates of the same bacterium were obtained from the corn breeding plots of the North Carolina Agricultural Experiment Station Test Farm at Clayton and also from a field of an inbred, N. C. 7, near Winterville, North Carolina.

Reports of bacterial stalk rot diseases of corn have been made by Ark (1), Boewe (2), Johnson et al. (3, 4), Lanza (5), Ludbrook (6), Prasad (7), Rosen (8, 9, 10), Sabet (11), Stanley (12), and Stanely and Orton (13). However, preliminary results obtained in several routine biochemical tests indicated that the bacterium isolated from diseased plants differed from other bacteria previously associated with stalk rot diseases of corn. This study was initiated to determine 1) whether the bacterium isolated from diseased corn plants was the primary pathogen, 2) whether this bacterium could also cause a typical soft rot of plants other than corn, and 3) whether it could be distinguished from the other stalk rot and soft rot bacteria previously described.

SYMPTOMATOLOGY

The most striking characteristic of the disease is the breaking over or toppling of plants above the fourth or fifth node (Fig. 1, A, B). This symptom differs from that described by Rosen (8, 9, 10) for the stalk rot disease caused by Erwinia dissolvens (Rosen) Burk. which usually attacks the stalk close to the ground line. Diseased leaf and stem tissues become water soaked and light tan to brown. The pith is disintegrated and often reduced to a slimy consistency; water soaked streaks extend short distances into the lamina of the leaf. Shredding of leaf tissue at the juncture with the stem is common. In advanced decay only the vascular bundles remain intact. In addition, diseased plants have a distinctly unpleasant offensive odor.

[1] Grateful appreciation is expressed to Dr. A. Husain for assistance in the laboratory studies.

FIGURE 1. Diseased corn plants showing symptoms of stalk rot. A -- Typical symptoms of stem collapse; B -- stalk breaking at node showing discoloration.

MATERIALS AND METHODS

Initial inoculations were made with two isolates, one from plants obtained at McCullers, and the other from Winston-Salem. Subsequently these tests were repeated and confirmed using six additional isolates obtained from diseased plants at Clayton and Winterville, North Carolina.

Stock cultures were stored in test tube slants at 25° C under sterile mineral oil on a peptone-tryptone-yeast extract medium. The methods employed in preparing culture media and completing standard biochemical tests followed the procedures specified in the Manual of Methods for the Pure Culture Study of Bacteria (Society of American Bacteriologists).

In preparation of the inoculum for pathogenicity tests, isolates were streaked on nutrient dextrose agar plates. After 48 hours, five loops of bacteria were removed from the plates and suspended in 3 ml of sterile distilled water. Six week-old Golden Bantam corn plants approximately 3 feet in height growing in a greenhouse soil bed were inoculated by placing a

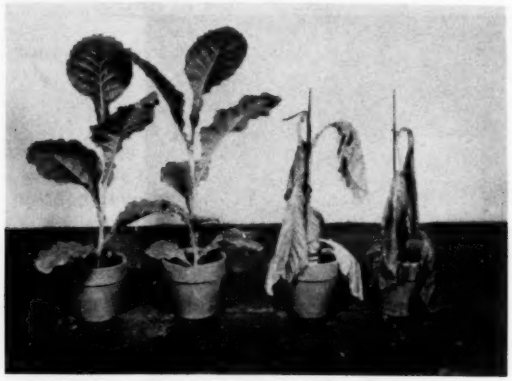

FIGURE 2. Right: two Dixie Bright 101 tobacco plants showing symptoms of wiliting 7 days after inoculation with the corn stalk rot bacterium. Left: two non-inoculated check plants.

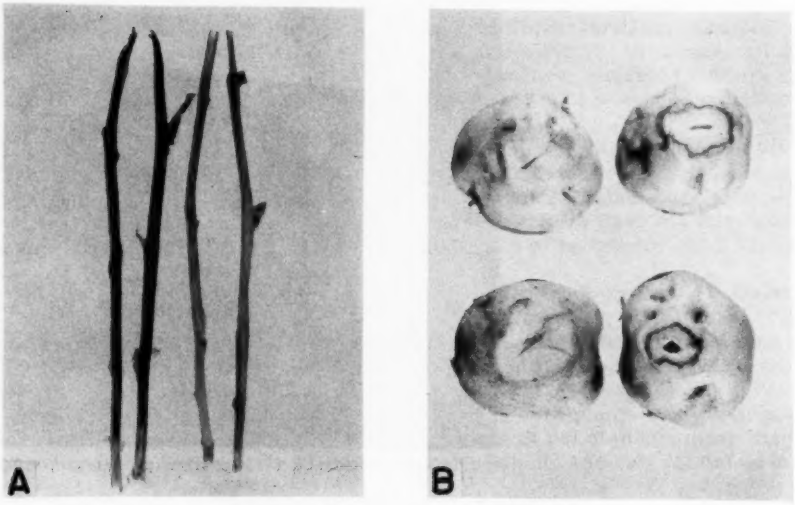

FIGURE 3. A -- Right: sections through stems of non-inoculated check plants. Left: longitudinal sections of 2 stems of Dixie Bright 100 tobacco plants showing decay of pith caused by the corn stalk rot bacterium. B -- Right: Irish potato tubers 48 hours after inoculation with the corn stalk rot bacterium. A dark brown to black discoloration borders the zone of soft decay. Left: non-inoculated tubers.

few drops of the bacterial suspension in a needle puncture made at the third or fourth node above ground level. A moist pad of sterile cotton was placed over the point of inoculation and taped in place. Check plants were treated in the same way except that sterile water was substituted for the bacterial suspension.

Dixie Bright 101 tobacco (Nicotiana tabacum L.) plants growing in 6-inch pots and about 40 cm in height were inoculated in the following manner. The apical portion of the stem was removed with a sharp scalpel at right angles to the stem, a single drop of bacterial suspension was placed on the cut surface and a sterile needle forced several times through the drop and into the pith to a depth of about 1 cm. Four to six plants were inoculated with each isolate. All inoculation tests of corn and tobacco were repeated three times and re-isolations were made from infected plants.

Irish potato (Solanum tuberosum L.) tubers that were used in laboratory inoculations were soaked for 10 minutes in a solution of mercuric chloride (1:1000), washed in sterile water and placed in glass or plastic moisture chambers. A slice of tissue was removed from the upper side of each tuber and a 1/2 inch cut was made into the exposed surface. One loop of a bacterial suspension was placed in the cut. Inoculations were similarly made in fleshy portions of carrot (Daucus carota L. var. sativa DC.) roots, cabbage (Brassica oleracea L. var capitata L.) leaves, squash (Cucurbita pepo L. var. melopepo (L.) Alef.) fruits, onion (Allium cepa L.) bulbs, and cucumber (Cucumis sativus L.) fruits. Four to six of these were inoculated with each of four isolates and all tests were repeated three or more times.

RESULTS

Pathogenicity -- All isolates of the corn stalk rot bacterium proved to be highly pathogenic, causing a rapid soft rot of parenchymatous tissue on all inoculated plants or plant parts. Water-soaking and discoloration of tissues around the inoculation court on corn plants were evident 48 hours after inoculation. After 3 to 5 days, extensive invasion of stem tissues was evident and plants frequently toppled over. Symptoms in general resembled those observed in the field. The bacterium was consistently re-isolated from diseased portions of inoculated plants.

Inoculations on tobacco resulted in a rapid destruction of pith tissue; the decay frequently extended 12 to 20 cm below the cut surface. In general, the symptoms produced (Fig. 2; Fig. 3, A) were similar to those described for the hollow-stalk bacterium, Erwinia aroideae (Towns.) Holland (15). The leaves in the upper portions of the stem where the pith was destroyed became yellow and gradually wilted. Once the infection ceased spreading, however, axillary suckers developed at points on the stem below the area of decay and one or more new shoots frequently developed.

Symptoms of decay on parenchymatous tissue of potato tubers (Fig. 3, B) and carrot roots were essentially similar to those described for other soft rot bacteria. In general, the rate of development of decay was extremely rapid in all inoculated fleshy vegetables; at a temperature of 28° C, cucumbers, squash, carrots, and cabbage were often almost completely rotted within 12 hours.

Morphology and Physiology -- Following standard procedures, it was found that the bacterium was a motile, non-capsulated, short, Gram-negative rod with peritrichous flagella.

In a preliminary study of physiological characteristics, the bacterium produced indole, hydrolyzed starch, reduced nitrate, liquefied gelatin, hydrolyzed carboxymethylcellulose, and either did not grow or grew very poorly in a tryptone broth containing 5 percent sodium chloride. These results, sugar fermentation reactions, and inoculation studies indicate that the bacterium is probably a species of Erwinia. It is, however, different with respect to one or more physiological characteristics and pathogenicity to corn from the previously described species of Erwinia that cause soft rots of plant tissues (14). In order to prepare a complete description of this bacterium in comparison with other soft rot bacteria, a more detailed study of the physiological characteristics of the bacterium has been initiated.

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SESAME (SESAMUM INDICUM L.), A HOST FOR VERTICILLIUM ALBO-ATRUM REINKE & BERTH.[1]

John E. Chilton[2]

The search for new cash crops in the South and Southwest has created considerable interest in sesame (Sesamum indicum L.). The susceptibility of this crop to various diseases has already been reported by various workers.

The New Mexico Agricultural Experiment Station has grown numerous introductions of sesame for observation under Southwestern irrigated conditions. In 1955, the author observed many of the breeding strains growing on known Verticillium albo-atrum-infested soil. Severe disease symptoms occurred throughout the progeny planting. The symptoms were typical of Verticillium wilt, including the yellow mottling and epinasty of leaves, with considerable necrosis in the mottled areas. Defoliation in general was severe, with consequent stunting or even death occurring. Internally, brown streaks in the xylem tissue of petioles, stems, and roots were pronounced. Verticillium albo-atrum was successfully isolated from several plants, and Koch's postulates for proof of pathogenicity were satisfied, as described below.

Pure cultures of the V. albo-atrum isolate from sesame were added as a blended mycelium-sclerotium-spore water suspension to sterilized soil in 10-inch clay pots. Similarly, an isolate from cotton was added to other pots. The pots were then planted in four replicates with either sesame or cotton.

Table 1. Pathogenicity of Verticillium albo-atrum isolated from sesame and cotton.

Source of inoculum	Average number of days to first leaf symptom: In pots inoculated with: Cotton	Sesame	Hypodermic inoculation of cotton plants
Sesame	33	41	7
Cotton	30	45	7 1/2

In Table 1 are listed the average number of days to symptom development. No difference in time or severity of symptoms occurred between sesame or cotton isolates. Also included in the table are data from comparative hypodermic inoculations of cotton with both sesame and cotton isolates. Again no indication of pathogenic variability appeared.

This is apparently the first published record of Verticillium wilt of sesame in the United States. Previous records of the disease consist of one report of Verticillium dahliae Kleb. in Turkestan and central Russian Asia, and a questionable report of V. dahliae in either Europe or Asia[3].

The severity of the disease on sesame makes mandatory the careful selection of land for growing this crop in areas of V. albo-atrum infestations. General observations of infected sesame plantings in New Mexico indicate that date of planting might greatly influence the susceptibility of sesame to wilt. Additional work might reveal useful cultural methods for alleviating the disease.

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[1] Journal Series No. 113, New Mexico Agricultural Experiment Station, New Mexico College of Agricultural and Mechanic Art, State College, New Mexico.

[2] Assistant Plant Pathologist.

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ANGULAR LEAFSPOT OF TOBACCO IN FLORIDA

John H. Owen and Fred Clark[1]

Angular leafspot of tobacco, caused by Pseudomonas angulata (Fromme & Murray) Holland, was observed during May and June, 1957 in several fields of flue-cured tobacco growing near Newberry, Florida. The disease was particularly severe in one 9-acre field where it was first noticed on the lower 8 to 10 leaves of plants 3 feet tall.

The very early stage of infection appeared as irregular, black, watersoaked areas, and the infected tissues rapidly became necrotic, forming dark-brown angular lesions. The centers of these lesions often became light tan with dark-brown borders as they aged (Fig. 1). During rainy weather the necrotic tissues often fell out. A slight chlorosis was sometimes observed as a narrow yellow band around the lesion. The lesions ranged from 1 to 10 mm in diameter and frequently coalesced. involving large areas of the leaf blade.

FIGURE 1. Infected tobacco leaf showing angular lesions with light-tan centers and brown borders.

The disease had apparently become well established in the field and had continued to spread from April 30 through May 20, as the rainfall during this period was favorable for infection (Table 1). Heavy infection was very noticeable following a 3-inch rainfall on May 16, and the 4 consecutive days of light rainfall that followed aided disease development. The disease appeared to be checked from May 21 until June 3. The new growth developing during this period was fairly free of the disease, primarily because of low rainfall. Daily rains beginning on June 3 and continuing through June 10 totaled 5.77 inches, and a severe outbreak of the disease again occurred following heavy rainfalls of 2.25 inches on June 8 and 1.19 inches on

1 Associate Professor of Plant Pathology, College of Agriculture, and Associate Agronomist, Agricultural Experiment Station, University of Florida, respectively.

Table 1. Daily rainfall for Gainesville, Florida from April 15 to June 16, 1957.

Rainfall (inches)							
April		May		May		June	
15	.57	1	.06	17	.11	1	-
16	-	2	.07	18	.02	2	-
17	-	3	.20	19	.08	3	.03
18	-	4	.06	20	.02	4	.12
19	.55	5	.01	21	-	5	.97
20	.05	6	-	22	-	6	.95
21	-	7	-	23	.02	7	.19
22	-	8	-	24	-	8	2.25
23	-	9	-	25	-	9	1.19
24	-	10	.20	26	-	10	.07
25	-	11	.53	27	.05	11	-
26	-	12	-	28	-	12	-
27	-	13	.79	29	-	13	-
28	-	14	-	30	-	14	-
29	-	15	.12	31	.11	15	-
30	.45	16	3.00			16	-

June 9. The damage was considerable, and the market quality of the tobacco was greatly reduced.

Numerous isolations from diseased tissues consistently yielded the angular leafspot bacterium. The organism was probably brought in on contaminated seeds, as the disease was found to be present to a lesser degree in several other fields where plants had been obtained from the same seed beds.

Apparently this is the first report of angular leafspot from Florida; however, wildfire, caused by Pseudomonas tabaci (Wolf & Foster) Stevens, has been reported as causing damage to tobacco in the State.

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THE EFFECT OF ANTIBIOTICS ON PSEUDOMONAS GLYCINEA[1]

M. J. Kaufmann and D. W. Chamberlain[2]

Summary

Laboratory assays employing the paper-disk plate method demonstrated that 10 ppm of aureomycin, achromycin, and tetracycline inhibited the multiplication of Pseudomonas glycinea. Streptomycin was moderately effective at 100 ppm. In field tests, only streptomycin at 250 ppm gave satisfactory control of bacterial blight, but induced varying amounts of leaf chlorosis. The addition of manganese sulfate to the antibiotic solution prevented the leaf chlorosis but occasionally caused a "scorching" of the leaves. Since the chlorosis appears to do little or no damage, the risk involved in controlling it with manganese is not considered justified.

In 1954 a number of antibiotics were evaluated for their effectiveness against Pseudomonas glycinea (Coerper) Stapp, the cause of bacterial blight of soybean (Glycine max (L.) Merrill). There were two objectives: first, to determine whether antibiotics hold any promise as a practical control measure; and second, to determine their value as a research tool in maintaining bacterial blight disease-free control plants in field experiments where natural infection often creates a problem.

MATERIALS AND METHODS

The following antibiotics were included in the test: Hydrochlorides of achromycin and aureomycin, magnamycin, neomycin, tetracycline, streptomycin sulfate, and a streptomycin-neomycin mixture. A paper-disk plate method similar to the one described by Thornberry (2) was used. Plates were prepared in advance by pouring 15 ml of Wernham's Buffered P. D. A. into Petri dishes and allowing the agar to solidify. When an assay was run, agar at 45° C was seeded with a heavy suspension of Pseudomonas glycinea. Five ml of the seeded agar was poured over each of the prepared plates to provide a uniform layer. Filter paper disks were then placed on the surface of the agar and 0.09 ml of the antibiotic solution was applied to each immediately. The zone of inhibition around each paper disk was measured after 24 hours. Antibiotics were assayed at 10, 100, 1000, and 10,000 ppm of active ingredient.

The field experiments comprised four replicates per treatment, each replicate consisting of a treated and an untreated row of Adams soybeans. Each antibiotic was applied at two concentrations, 100 ppm and 250 ppm of active ingredient. Treatments were applied weekly and the plants were inoculated with P. glycinea 24 hours after each treatment. Inoculations were made with a Hudson power sprayer operated at a pressure of 125 psi. The nozzle was adjusted to deliver a fine stream of water with sufficient force to watersoak the leaves with inoculum. An additional block of plants was added to test the effect of manganese on streptomycin-induced chlorosis, comparing streptomycin (250 ppm) alone, and in combination with 0.1, 0.2, and 0.3 percent manganese sulfate. Triton B-1956 was used as a wetting agent for all treatments at the rate of 2 ounces per 100 gallons.

RESULTS

In the laboratory assays, aureomycin, achromycin and tetracycline at 10 ppm inhibited the multiplication of Pseudomonas glycinea. Streptomycin was moderately effective at 100 ppm, but was ineffective, alone or in combination with neomycin, at 10 ppm. The other antibiotics were ineffective at concentrations below 1000 ppm. Magnamycin had no inhibitory effect

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[1] Publication No. 305 of the United States Regional Soybean Laboratory, Urbana, Illinois.

[2] Research Assistant, Department of Plant Pathology, University of Illinois, and Plant Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, respectively, cooperating.

Grateful acknowledgment is made to the following firms: Charles Pfizer Co. for magnamycin, streptomycin, terramycin, and tetracycline; Lederle Laboratories for achromycin and aureomycin; the Upjohn Company for neomycin, and Merck and Company for streptomycin.

on P. glycinea at any level tested.

The four most promising antibiotics from the laboratory assay were tested in the field in 1954 to determine their effectiveness as a control for bacterial blight. One week after application of the first treatment a yellow chlorosis was noted on the leaves of soybeans treated with streptomycin at 250 ppm. Rosen's (1) description of the role of manganous ions in preventing streptomycin-induced inhibition of growth in oat coleoptiles suggested an additional experiment to test the effect of manganese on streptomycin chlorosis in soybeans.

The plants were observed weekly and final evaluation of the treatments was made 10 days after the fifth treatment.

Streptomycin at 250 ppm gave approximately 90 percent control of bacterial blight. At 100 ppm about 50 percent control was obtained, while achromycin at 250 ppm was slightly less effective. The other treatments had no apparent effect on the development of bacterial blight.

The yellow chlorosis induced by streptomycin at 250 ppm was reduced appreciably by the addition of 0.2 percent manganese sulfate and completely eliminated at 0.3 percent. The control of bacterial blight with streptomycin plus manganese sulfate was equal to that with streptomycin alone.

In 1955 tests were continued with streptomycin alone and in combination with manganese sulfate. Inoculations with P. glycinea were made 3 days after applying the antibiotic. An early natural epidemic of bacterial pustule in the experimental plots necessitated discontinuance of the experiment after the third antibiotic treatment. Up to that time no blight infection had been found on the treated plants. Untreated plants showed light infection. Replicates treated with 250 ppm streptomycin alone showed less leaf chlorosis than in 1954. Again, the addition of 0.3 percent manganese sulfate to the streptomycin solution completely suppressed the leaf chlorosis, confirming the results of the previous year.

A third trial, in 1956, again confirmed the effectiveness of streptomycin in controlling bacterial blight. Unfortunately, soybean plants treated with the antibiotic plus manganese sulfate developed an appreciable amount of leaf "scorching". Since the manganese sulfate used in 1954, 1955, and in 1956 was from the same lot of the chemical, there is no valid explanation for this type of leaf damage in 1956.

The chlorosis induced by streptomycin alone apparently did no permanent damage and the leaves eventually recovered their normal color.

DISCUSSION

The effectiveness of streptomycin against Pseudomonas glycinea in the field and the failure of the other antibiotics to control bacterial blight again emphasizes the difference between laboratory assays of bactericidal or bacteriostatic properties and their use in the control of plant diseases. Although streptomycin ranked below three other antibiotics in the laboratory tests, it was the only one to give satisfactory disease control in the field. Its use as a practical control measure at present is debatable economically. However, streptomycin does offer the research worker a valuable tool with which to protect control plants against accidental infection with bacterial blight when such controls are essential for an experiment.

On the basis of 3 years' observations, the soybean plant apparently recovers from the streptomycin-induced chlorosis with no permanent damage. Moreover, the severity of the chlorosis varies considerably from year to year, and even from one application to the next. Although manganese applied with the antibiotic spray suppressed chlorosis completely, the unexplained burning of the leaves in 1956 is sufficient to question its usefulness. It seems preferable to use streptomycin alone when control of bacterial blight is needed until more information concerning its relationship with manganese is available.

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TWIG CANKER OF SWEETGUM

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A stem canker of sweetgum (Liquidambar styraciflua L.), found near Stoneville, Mississippi, in 1952, occurs frequently on seedlings and lower branches of larger trees. In localized areas seedlings of poor vigor or under shade are killed. Botryosphaeria ribis Gross. & Dug. was commonly found fruiting on the dead portions of the cankers and was consistently isolated from canker margins. Tubercularia vulgaris Tode was found on a few recently killed sweetgum twigs.

B. ribis has been reported to cause canker and dieback of at least 50 woody plants (2). Recently Pirone (1) reported briefly on inoculation experiments with sweetgum in New York City in which he showed that B. ribis is capable of causing infection. No such experiments have been reported on sweetgum in other areas.

To determine whether B. ribis and T. vulgaris are pathogenic on sweetgum twigs, some 6- to 8-year-old sweetgum seedlings in an old field near Stoneville, Mississippi were selected for inoculation. The trees ranged from 1/4 to 2 inches d. b. h. and 5 to 11 feet in height. In 1952, 20 trees were inoculated with B. ribis and 20 with T. vulgaris, while 10 were used as checks. Each tree was inoculated at five points: on the leader, and on four separate lateral branches. A piece of fungus from a culture tube was placed in a small wound made at each inoculation point. Sterile agar was used in the check wounds.

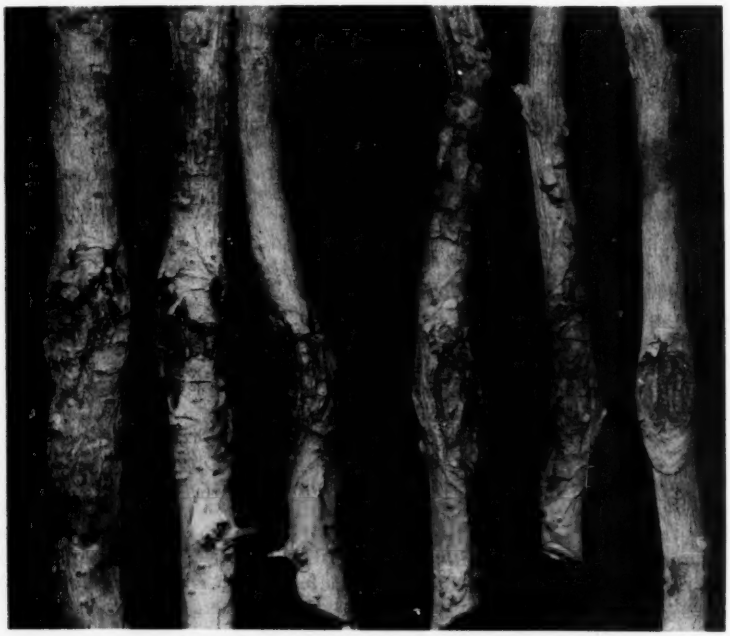

FIGURE 1 Cankers on sweetgum twigs 4 years after inoculation with Botryosphaeria ribis. (About 3/4 size.)

1Stationed at the Delta Research Center, Southern Forest Experiment Station, Forest Service, United States Department of Agriculture. The Delta Research Center is maintained in cooperation with the Mississippi Agricultural Experiment Station at Stoneville, Mississippi. It is partially financed by the Southern Hardwood Forest Research Group.

One year after inoculation, all of the check twigs and those inoculated with T. vulgaris were uninfected and healed. At that time 81 percent of the smaller twigs (less than 0.2 inch in diameter) inoculated with B. ribis were infected, as compared with 45 percent of the larger twigs (over 0.3 inch in diameter). By 1956 one of the trees inoculated with B. ribis had died of suppression. Of the remaining 76 lateral branches inoculated with B. ribis, 83 percent had cankers, and 57 percent of the cankered branches were dead (Fig. 1). Of the 19 leaders, 36 percent were infected, but none were killed. B. ribis was re-isolated from all of the infected twigs.

These data show that B. ribis can produce cankers and kill twigs when it gains entrance through wounds. The fungus, however, appears to be a weak pathogen, and so far seems harmful only to shaded or weakened twigs and branches.

Literature Cited

1. PIRONE, P. P. 1952. A bark canker disease of London plane. (Abstract.) Phytopathology 42: 16.
2. WESTCOTT, C. 1950. Plant disease handbook. 746 pp., illus. New York.

SOUTHERN FOREST EXPERIMENT STATION, FOREST SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE

A NEW DISEASE ON WESTERN FLOWERING DOGWOOD

Lindsay B. Loring[1]

An apparently undescribed disease affecting the flower bracts of western flowering dogwood, Cornus nuttallii And., has been under observations for three springs in one location of the Willamette Valley of Western Oregon.

Dull yellowish brown lesions mar the beauty and cause some distortion of the creamy white bracts. The irregular-shaped necrotic areas vary from about 2 to 15 mm in length and are roughly parallel with but not delimited by the veins of the bracts. The most common size of lesions observed has been approximately 4 x 8 mm in area. Under humid conditions spores may accumulate in abundance on either surface and the masses can be seen with a hand lens as numerous small flecks. The organism associated with and apparently the causal agent of the malady is a Fusicladium. A review of the available literature has not revealed a Fusicladium associated with species of Cornus.

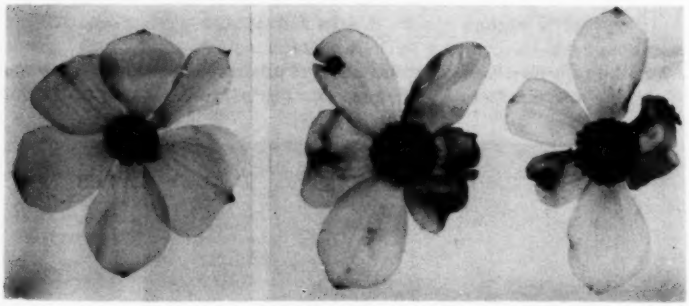

FIGURE 1. Normal healthy dogwood bloom on left. Blooms at center and to the right with bracts distorted and spotted with the Fusicladium disease.

A rapid examination was made in April 1957 of 103 small to large dogwoods in partially logged-off and second growth timber adjacent to U. S. Highway 99 between Chemawa and Wilsonville. Only 9 percent of the trees revealed from a trace to about 5 percent of the blooms to be affected.

Three collections of dogwood blooms made May 5, 1957 on the west slope of the Coast Range between Florence and Blachly in Lane County along State Highway No. 36 each yielded Fusicladium. Severity in each of these locations was limited to a trace.

DIVISION OF PLANT INDUSTRY, OREGON DEPARTMENT OF AGRICULTURE, SALEM, OREGON

[1] Plant Pathologist.

CYTOSPORA CANKER OF ROCKY MOUNTAIN DOUGLAS-FIR

Ernest Wright[1]

Recently Waterman[2] summarized the literature on the occurrence of Valsa (Cytospora) cankers on coniferous species in the United States. The evidence indicates that Cytospora is a secondary disease that commonly infects conifers weakened by drought, fire, insects, or any unfavorable environmental condition. This paper gives some corroborative information from unreported studies on the so-called pitch-girdle canker of Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco).

HISTORICAL

Roeser[3] was first to report the pitch-girdle canker, which had been present for several years in stands of Douglas-fir, on the eastern edge of the Rocky Mountains near Sedalia, Colorado. However, the identity of the disease was not established at that time. Although Valsa was later observed fruiting in association with the canker, it was not considered the causative fungus.

In the mid-thirties, the writer was assigned additional studies of Roeser's pitch girdle. It was necessary to determine the cause of the disease, its distribution, and the reasons for its rapid development.

PITCH-GIRDLE STUDIES

Isolations and inoculation tests were made with the Cytospora commonly associated with pitch girdle. Typical cankers with heavy resin flow were artificially produced by the slit and cork-borer methods[4] (Fig. 1). Death of inoculated trees resulted from killing of the cambium and girdling. The Valsa stage failed to develop in any of these inoculations. Finally, the isolated fungus was identified by Waterman as C. kunzei Sacc.

FIGURE 1. A pitch-girdle canker resulting from inoculation of young Douglas-fir with Cytospora kunzei Sacc. Taken 3 years after inoculation, this photo shows the characteristic resin flow and scaly bark that frequently develops on naturally cankered trees.

[1] Senior Pathologist, Pacific Northwest Forest and Range Experiment Station, Forest Service, United States Department of Agriculture.

[2] Waterman, Alma M. 1955. The relation of Valsa kunzei to cankers on conifers. Phytopathology 45: 686-692.

[3] Roeser, J., Jr. 1929. The occurrence of "pitch girdle" in sapling Douglas-fir stands on the Pike National Forest. Jour. For. 27: 813-820.

[4] Wright, E. 1933. A cork-borer method for inoculating trees. Phytopathology 23: 487-488.

FIGURE 2. Natural stem infection of Douglas-fir by Cytospora kunzei 40 feet above the ground. The outer bark has been cut away to expose the necrotic cambium and better show the start of canker formation.

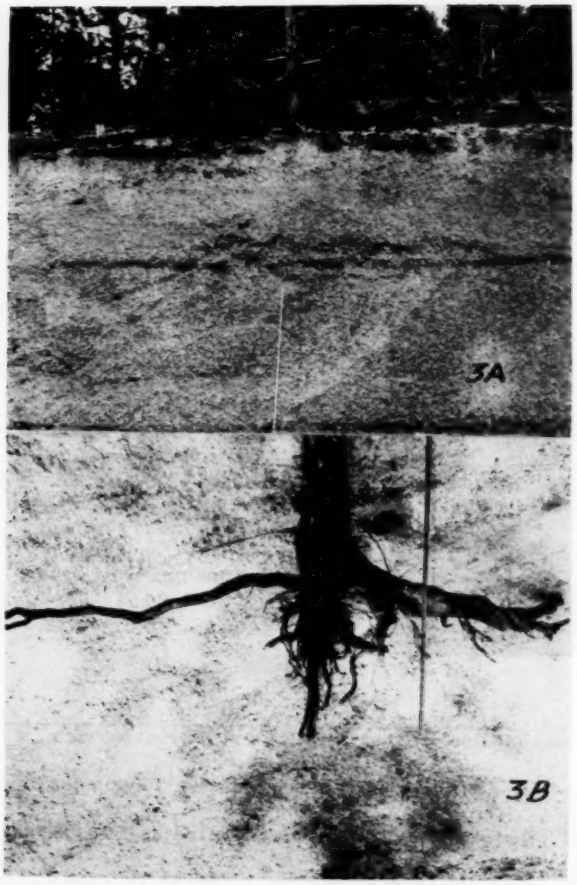

FIGURE 3. The soil mantle is thin and underlaid with granitic gravel many feet thick in much of the eastern part of Colorado's Rampart Range. This results in poorly developed root systems and consequent lowering of resistance to disease during periods of drought. A, Road cut in a 30-year-old Douglas-fir stand. Soil layer is about 1 1/2 feet thick. The steel tape is 6 feet long. B, Root system of 30-year-old Douglas-fir. Note the short taproot and fairly extensive lateral roots. The steel tape is 3 feet long.

Inoculation tests showed that the Cytospora grew best under thin bark and only very slowly under thick bark. This characteristic probably accounted (at least partially) for its presence in the upper bole of old trees (Fig. 2). Roeser also reported the common infection of tree tops. He likewise observed that the occurrence of pitch girdle decreased with a decrease in stand density. Thinning, however, did not result in an expected decrease in disease. It was apparent that factors other than stand density were involved.

During the course of this study, C. kunzei cankers occasionally were found on isolated trees growing on obviously poor sites. In such situations the upper soil horizons were frequently very thin and generally underlaid with granitic gravel many feet thick. This resulted in poorly developed root systems (Fig. 3). Any pronounced deficiency in precipitation caused a weakening and a rapid reduction in tree growth, which subsequently led to infection and killing by C. kunzei.

United States Weather Bureau records show that during the five years preceding Roeser's studies (1917-1921), except for 1921, precipitation was noticeably deficient, especially during the growing season. Precipitation since 1940 has returned to near-normal in the eastern Rocky Mountains, and it is the writer's understanding that pitch-girdle cankers on Douglas-fir are now much less common than in former years.

PACIFIC NORTHWEST FOREST AND RANGE EXPERIMENT STATION, FOREST SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE

NEW AND UNUSUAL RECORDS OF PLANT DISEASE OCCURRENCE

WHITE ROT OF ONION IN NEW YORK

By G. D. Lewis[1]

White rot of onion, caused by Sclerotium cepivorum Berk., was found in two muckland onion fields near Florida, New York, in June, 1957. A dense white mycelium containing the sclerotia of S. cepivorum was found on the surfaces of infected bulbs. Approximately 30 percent of the plants in the affected areas were lost. Another 20 percent stopped growing prematurely. Conversation with the grower indicated that this disease has appeared in one of the two fields in previous years.

This appears to be the first time that white rot has been found in New York.

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NEW HOSTS OF MACROPHOMINA PHASEOLI IN PERU

By C. Bazán de Segura

In 1950, for the first time in Peru, the fungus Macrophomina phaseoli (Maubl.) Ashby was isolated from seedlings of sour orange. These plants were sent from Chiclayo (North Coast of the country).

Later, in 1955, we found the same fungus on potato tubers harvested at the Agricultural Experiment Station La Molina. These tubers were very badly injured by the fungus.

At the end of the past year and in the first months of 1957, we determined the presence of this parasitic fungus in the Central Peruvian Coast, on the following new hosts: bean, castor-bean, and olive-tree (3 years old). It is possible that the meteorological conditions of the last years in Peru have been highly favorable to infection by M. phaseoli.

In Peru so far, M. phaseoli has been determined on the following hosts:

1. Sour Orange (Citrus aurantium)
2. Potato (Solanum tuberosum var. andigenum)
3. Bean (Phaseolus vulgaris)
4. Castor-Bean (Ricinus communis)
5. Olive-tree (Olea europaea)

On all these hosts, the losses produced by this fungus were very serious.

ESTACION EXPERIMENTAL AGRICOLA DE LA MOLINA, PERU

THE BURROWING NEMATODE FOUND ON ORNAMENTAL BANANA PLANTS AT BATON ROUGE, LOUISIANA

By Leigh S. Whitlock[1]

Banana plants grown as ornamentals about buildings in the Baton Rouge, Louisiana, area were found to be infected with the burrowing nematode, Radopholus similis (Cobb 1893) Thorne 1949. Of the nine sites sampled in a preliminary survey, three yielded moderate numbers of both males and females of R. similis. This nematode has previously been reported from Louisiana from sugarcane roots[2], and from Pothos and Philodendron in two ornamental nurseries[3].

UNITED STATES DEPARTMENT OF AGRICULTURE, AGRICULTURAL RESEARCH SERVICE, CROPS RESEARCH DIVISION, BATON ROUGE, LOUISIANA
[1] Assistant Nematologist, United States Department of Agriculture, Agricultural Research Service, Crops Research Division, Baton Rouge, Louisiana.

[2] Rands, R. D. 1930. Proc. 3rd Cong. Internat. Soc. Sugar Cane Technol. Soerabai. 7-9: 119-131.

[3] Records of the Nematology Section, Beltsville, Maryland, and Report of the Burrowing nematode Survey in Louisiana March 13 to April 13, 1956, United States Department of Agriculture, Plant Pest Control Branch.

PHYTOPHTHORA CINNAMOMI RANDS APPARENTLY PARASITIC ON TWO JUNIPER VARIETIES

By Lindsay B. Loring and Harriet Smithson[1]

Earlier reports of observations and experimental testing in Oregon had indicated that junipers might be completely resistant to attack by Phytophthora cinnamomi Rands. Recent observations of typical Phytophthora symptoms in a nursery, and the isolation of the pathogen from the advancing edge of discolored tissue of the stems, seem to offer strong evidence that two varieties of junipers are susceptible to P. cinnamomi.

One of the junipers was labeled as Juniperus sabina tamariscifolia and appeared to compare well in foliage with a correctly identified specimen from Arnold Arboretum of Harvard University.

The foliage of the other plant, which was considered to be an improved horticultural selection of J. chinensis sargenti, did not closely resemble the foliage of this variety sent from Arnold Arboretum.

Although the identification of these junipers is open to question it is significant that two varieties have been found to be infected with P. cinnamomi.

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[1] Plant Pathologist and Pathological Aid, respectively.

CORRECTIONS

REPORTER for July 15 (Vol. 41, No. 7), page 595. In Table 1, the L.S.D. values should read:

" L.S.D. .05	6.1	2.9	5.4
L.S.D. .01	8.1	3.9	7.2"

This was a calculation error on the part of the author, but it does not change the text and interpretations of the paper.

REPORTER for July 15 (Vol. 41, No. 7), page 630. Under Figure 3, Phacidiopycnis is misspelled.

REPORTER for July 15 (Vol. 41, No. 7), page 643. Figure 1 should read "Timber rot of tomato." instead of "Tuber rot of tomato."

IN THIS ISSUE (Continued).

A species of Fusicladium was found to be the cause of a disease of the flower bracts of western flowering dogwood in Oregon, according to LINDSAY, B. LORING, page 810.

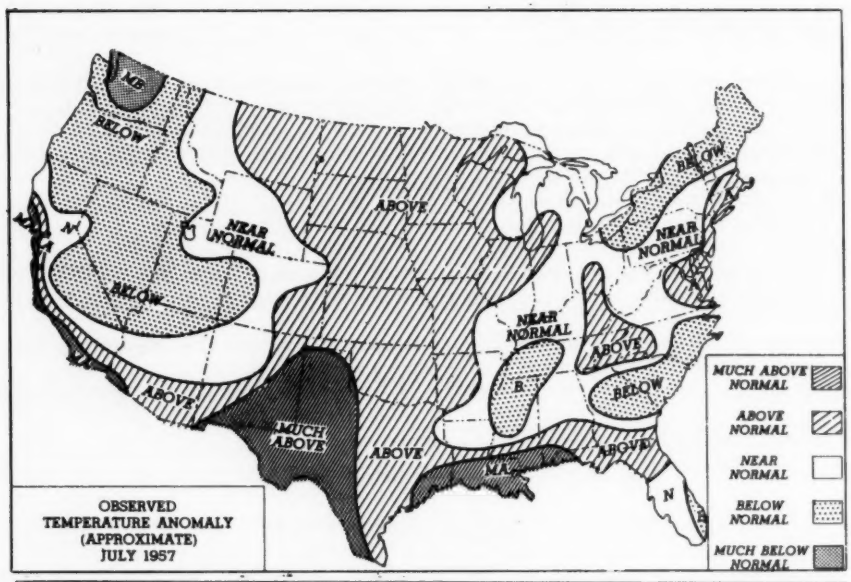
ERNEST WRIGHT reports that Cytospora canker of Douglas fir is a secondary disease affecting weakened or injured trees, page 811.

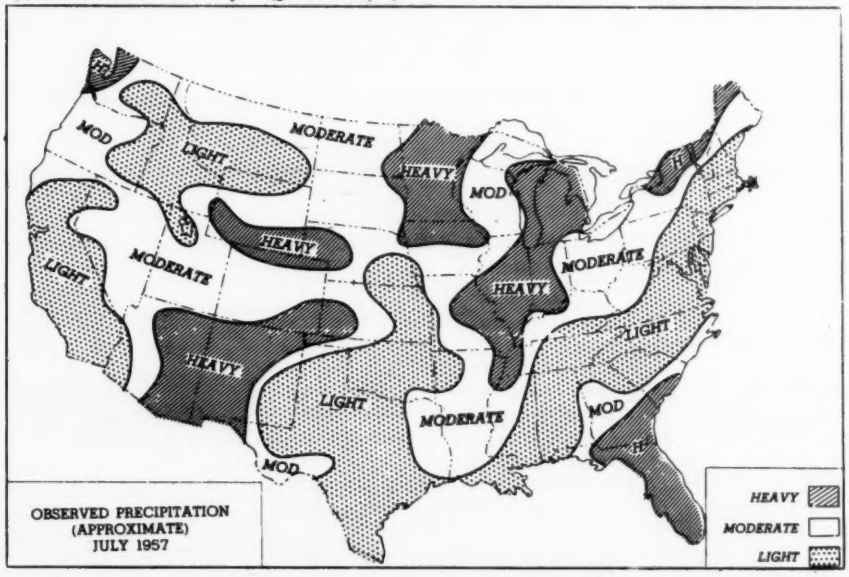
Unusual Records, page 814: White rot of onion in New York, by G. D. LEWIS; New hosts of Macrophomina phaseoli in Peru, by C. BAZAN de SEGURA; Burrowing nematode on ornamental banana in Louisiana, by LEIGH S. WHITLOCK; Parasitism of Phytophthora cinnamomi on juniper, by LINDSAY B. LORING and HARRIET SMITHSON.

Some corrections, page 815.

July weather, page 816.

Preparation of articles for the Reporter, page 817.





The terms used in the accompanying maps, which define the ranges of temperature and precipitation, are numerical class limits. These are based on a statistical analysis of past records through which is determined the normal frequency of occurrence of temperatures and precipitation at various times of the year for different locations. For temperature the classes above, below, and near normal are so defined that they each normally occur one-fourth of the time; much above and much below normal, one-eighth of the time. Precipitation is depicted in terms of light, moderate, and heavy, each class normally occurring one-third of the time and thereby having equal probability of occurrence. These maps graphically represent only the general trends and give the country's weather picture at a glance. For quantitative studies, where monthly mean temperatures and actual precipitation records are needed for a given time and place, other publications of the Weather Bureau should be consulted. P. R. M.

MANUSCRIPTS FOR THE PLANT DISEASE REPORTER

(See also PDR 35: 121-122; 36: 214, 494)

(1) GENERAL: The Reporter page measures 9 inches long with the heading or 8 3/4 inches for the text part, by 6 inches wide. The copy is typed on a larger page, 11 1/4 inches of text or 12 inches overall in length by 8 inches in width, and reduced 25 percent in the photographic process of reproduction. Illustrations or tables larger in either dimension will take a correspondingly greater reduction. Only one size of type is available for text, footnotes, or tables.

(2) MANUSCRIPTS should be the original ribbon copy, not carbons, clearly typed and double-spaced throughout, including tables, footnotes, and bibliographies. (Note -- only one copy is needed.) Footnotes should be typed at the bottom of the page.

(3) ABSTRACTS are requested for all except very short articles. (PDR 38: 316).

(4) CAUSES OF DISEASES should be named. For bacteria, fungi, nematodes, etc., give the Latin name of the organism; for viruses either or both the accepted common name of the virus or a Latin name if you prefer it and there is one; for non-parasitic diseases state the causal factor if it is known. If the cause of a disease has not been determined say so. (PDR 36: 494).

(5) LITERATURE REFERENCES should be given in alphabetical order and numbered for citation in the text. We follow the AIBS suggestion of placing the year of publication after the author's name. Please check your titles carefully since we cannot do it for you. Be sure that text citations and bibliography agree; that foreign-language references are correct; that number or month is cited for periodicals that are not paged consecutively throughout the volume.

(6) NAMES OF FUNGICIDES should be given according to the suggestions that will be published in Phytopathology before long, and later repeated in the Reporter. In the meantime see PDR 35: 122.

(7) ILLUSTRATIONS should be sent to us unmounted. To prevent mistakes, write figure numbers on the back, and mark the top of each print when necessary. A sketch can show a preferred arrangement but please keep in mind page size, shape, and standard reduction (see above under General), and remember that figure titles and legends are part of the page. Lettering should be clear and large enough to be legible after reducing. Drawings, maps, and graphs can be photographs or originals, but should be finished and ready for reproduction, not just sketches.

(8) TABLES should be carefully thought out with particular attention to the Reporter's limitations in reproduction. Make titles and headings definite and self-explanatory. Designate footnotes in tables with superscript lower-case letters. Be sure that text discussion agrees with the data in the table. Do not abbreviate names of crop varieties.

(9) REPRINTS cannot be supplied since there is no way in which we can be reimbursed. However,

(10) The MULTILITH PLATES from which reprints can be made will be sent if requested at the time the article is submitted. The press size of these plates used for the Reporter is designated as small -- maximum image 9 1/2 by 13 inches, maximum paper size 9 3/4 by 14 inches -- for Model 1250. Most of the Experiment Stations have this type of multilith machine.

(11) We are the official correspondents in the United States of the PLANT PROTECTION BULLETIN published monthly by the United Nations Food and Agriculture Organization. We would appreciate it if plant pathologists would send us suitable material from time to time for inclusion in our monthly summary reports. Please mark it clearly so that we will know it is intended for the Bulletin. (See PDR 36: 492; 37: 637) -- P. R. M.

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